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JOURNAL

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ROYAL MICROSCOPICAL SOCIETY

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TRANSACTIONS OF THE SOCIETY.

I.—THE PRACTICAL APPLICATION OF PHASE-CONTRAST TO THE BIOLOGIST'S MICROSCOPE.

By E. WILFRED TAYLOR, *C.B.E.*, F. Inst. P., F.R.M.S.

FIVE PLATES AND SIX TEXT-FIGURES.

INTRODUCTION.

IN the past the biologist has generally resorted to differential staining as a means of rendering visible slight non-homogeneities in his preparations. When such treatment was impracticable, as in the case of living cells, the alternatives were to study the out-of-focus image, to illuminate the specimen with very narrow pencils (with a consequent loss of resolution), or to use dark-ground illumination. Phase-contrast offers a means of converting slight changes of refractive index (with the consequent change of wave-front) into corresponding changes of amplitude. The method possesses the advantages that the object is accurately focused, that the full aperture of the objective is used, and that the eye is particularly sensitive to changes in contrast.

It also makes possible for the first time the detailed study at full aperture of transparent living tissue in place of the usual stained preparations, which may have undergone considerable modification in the course of processing.

HISTORICAL.

The use of a phase plate or "Z disc" to advance or retard the direct rays going to form an image with respect to the diffracted rays was first proposed by Zernike (1934), and in the original description the method was suggested as a means of greatly increasing the sensitivity of the knife-edge test as applied to the mirrors of reflecting telescopes. In a very practical paper by Burch

(1934), the Z test was applied to a decentred ellipsoid, and photographs were given of the consequent "Zernike fringes."

Thus matters remained for a short time with attention directed to astronomical reflectors, until Zernike (1935) turned his attention to the microscope and showed that the principles of phase-contrast could be equally well applied, not, in this case, to the testing of the objective but to the transmutation of a change of phase produced by the object to a change of amplitude in the image.

Professor Zernike's method was taken up by the firm of Carl Zeiss (German patent, 1932), and a very practical and well illustrated contribution on this subject by Kohler and Loos (1941) next appeared in Germany. This was followed by a further contribution by Zernike (1942) in which an analysis was made of the light distribution in the field of view of a phase plate illuminated by different methods.

A further addition to the literature on the subject was made by Burch and Stock (1942), who not only described the practical advantages of phase-contrast in detail but showed how, with a little ingenuity, a standard microscope could be converted to this method of observation.

Reference should also be made to contributions by Richards (1944) and Linfoot (1945), and to papers by Helen Jupnik (1944) and H. Osterberg (1944) in the U.S.A.

THE METHOD AS APPLIED TO THE MICROSCOPE.

In known systems for phase-contrast microscopy, the image of an illuminated diaphragm placed between the source of light and the substage condenser is formed between the objective and its image plane, and in this plane is placed a transparent phase-retarding plate which is such that a relative retardation of approximately $\lambda/4$ is produced between the phase of the light passing through the image of the clear portion of the condenser diaphragm directly to the focal plane and that which has been subsequently diffracted by the object.

The condenser diaphragm is preferably in the form of an annulus, for reasons of symmetry, and is mounted co-axially with the condenser. The corresponding phase plate must then consist of a phase-changing annulus coinciding in dimensions and position with the image of the condenser annulus. The phase plate is usually constructed of glass, and its phase-changing properties are obtained either by etching away a portion of its surface or, preferably, by depositing a suitable thickness of transparent material upon the surface in order to produce the required retardation, the essential feature being an optical path difference of approximately $\lambda/4$ between the two areas corresponding to the clear and opaque areas in the condenser annulus.

The phase-contrast effects in the image field are produced by interference between light which passes directly through the condenser annulus, the object, and the annulus in the phase plate and light diffracted by the object which passes through the remainder of the aperture of the phase plate. Since the latter is often weak there may be a marked difference in amplitude between the two interfering beams and consequent low contrast.

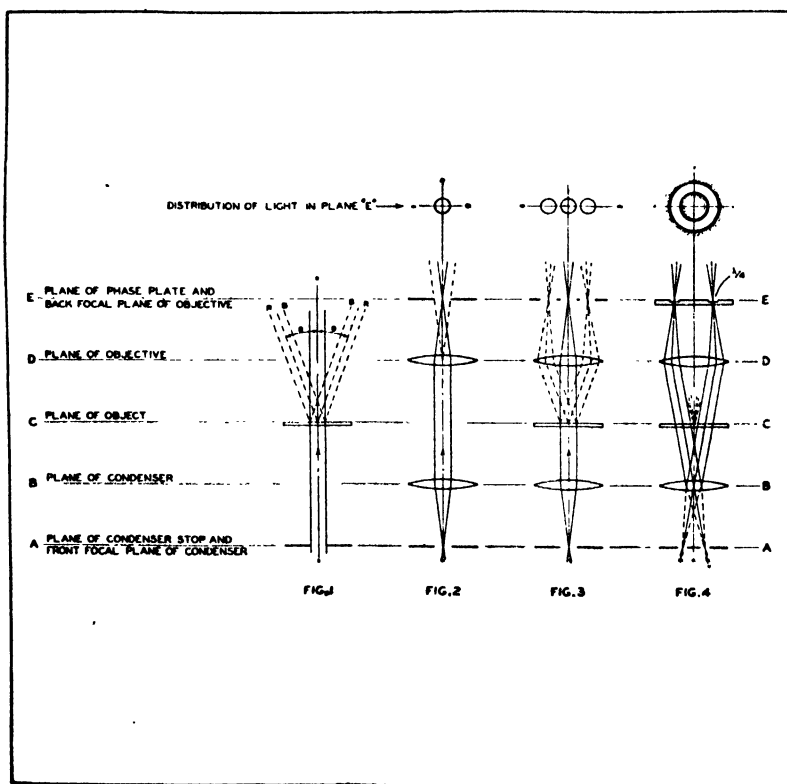
Two types of phase-contrast image may be produced. The so-called "positive" type, in which the annulus has a shorter optical path in relation to the rest of the plate, and the "negative" type, in which these conditions are reversed. Different types of object, or even different parts of the same object, may demand differing optical densities of phase annulus in order to secure maximum contrast.

THE GENERAL THEORY.

A rigid proof of the optical theory involved in phase-contrast is beyond the powers of the author, but it may be possible to move step by step towards an understanding of what is involved if we first confine our attention to regular structures.

The object is to transform slight non-homogeneities in an object into corresponding changes of amplitude or contrast in the image, and it will be found that this involves a change of phase which must be introduced between the objective and the image plane and in such a manner that two wave-fronts are formed in a condition to interfere with one another.

If a diffraction grating (C, fig. 1) is normally illuminated by parallel light, some of the luminous energy will be directly transmitted and some will be diffracted to either side to form the well-known spectra of the several orders. Here we need only consider those of the first order which differ in optical



Figs. 1-4.

path by λ and make angles θ with the direct rays. Since each colour is distributed strictly according to its wave-length, the after-images will be characterized by the spectral colours, blue towards the axis and red to the outside, but let us make a further simplification and assume that the light is monochromatic. The incident light has now been split into three groups of parallel rays, the angle θ depending only on the spacing of the rulings, which are assumed to be normal to the plane of the figure and the wave-length of the light.

In figs. 2, 3, and 4 are represented the illuminating and imaging system of a normal microscope, and the following planes are indicated following the path of the light :

- A, the plane of the condenser stop and the front focal plane of the condenser.
- B, the plane of the condenser.
- C, the plane of the object.
- D, the plane of the objective.
- E, the plane of the phase plate and the back focal plane of the objective.

As, in fig. 2, the condenser stop is mounted in the front focal plane of the condenser, the pencils of rays between it and the objective will be parallel, and an image of the condenser stop will be formed in the back focal plane of the objective under these conditions. All light proceeding to the focal plane must now pass through this image of the stop.

On introducing the grating into the object plane (C, fig. 3), it will produce the effects already noted, and, since we have now to consider three convergent pencils of rays, three images will be produced in the plane E. On removing the eyepiece and peering in, we shall see the white central image of the condenser stop and on each side the characteristic diffractive image of the first order. It is clear that in this plane it is possible to discriminate between the direct and the diffracted images, as indeed Abbé did in the course of his classical experiments.

At this stage it is of interest to consider what will happen if the grating is moved by half the pitch, so that the clear portions occupy the position previously occupied by the rulings. We know that the diffraction spectra will remain and we know also that they will disappear if the rulings are removed. Clearly, then, the diffraction spectra must change phase by $\lambda/2$ as a result of this movement, which in fact they do.

If the circular stop is replaced by an annulus (A, fig. 4) then the after-images in the plane E will also be of this form, and, for reasons to be given later, this form is preferred for the present purpose. In any case, a symmetrical form is desirable in order to secure uniform resolution over the field of view.

If the grating is removed and replaced by an object possessing slight non-homogeneities, we shall, on withdrawing the eyepieces, see an annular image surrounded by a faint halo of light both on the inside and outside. These diffuse halos are produced by diffracted light, while the image of the annulus is produced by direct or undeviated rays, and, in the absence of regular scattering in the object, do not form recognizable after-images.

In the plane E we now mount a phase plate to produce a relative retardation of $\lambda/4$ and of such a size as exactly to coincide with the image of the annulus.

All the diffracted rays will now undergo a retardation of $\lambda/4$ relatively to the direct rays, which are assumed to proceed unchanged to the focal plane of the eyepiece. Both wave-fronts have drawn energy from all parts of the object, and as interference takes place the darker areas in the image will correspond to areas of higher refractive index in the object. The sum total of light in the image plane will be unaffected, since there is no destruction of luminous energy, but only redistribution.

The diffuse halos do not possess the amplitude of the direct rays, with the result that the interference is only partial and a residue of direct light remains to veil the image. Conditions may be improved by introducing light-absorbing material into the phase-plate annulus.

The advantages of the annulus are two-fold: in the first place it permits differential treatment to be given to the diffracted rays on both the inside and outside of the annulus in relation to those transmitted by the annulus, and in the second place the exact coincidence between the phase plate and the image of the condenser stop is less important, resulting in the effective use of a greater proportion of the diffracted rays.

It remains to explain why the phase plate should produce a relative acceleration or retardation of $\lambda/4$ in order to obtain the maximum contrast in the image.

For the sake of simplicity, let us consider an object consisting of an alternating structure (fig. 5a) in which the shaded areas retard the wave-front. A ray of light may be represented both in phase and amplitude by a vector, a counter-clockwise turn of which indicates a retardation. After passing through the plate the vectors shown below may be represented by those shown above.

The retarded vectors above the shaded portions may be resolved at right-angles into two components y and z , of which the former is practically identical with the original vector before transmission (a'). The shaded areas have given rise to a new component, Z , which has been retarded by $\lambda/4$.

If these effects are now separated out, we shall have at b the effect of an almost uniform transparent plate, and, if the optical arrangements are as indicated in fig. 4, all the light transmitted through it will in the ordinary way pass through the image of the annulus in the plane E and give rise to even illumination in the image plane. The components Z will, however, give rise to diffraction spectra and will not pass through the image of the annulus.

If by means of a phase plate we now accelerate all the light passing through the annulus by $\lambda/4$ (which is equivalent to retarding the diffracted rays), we shall have the condition of things shown at c and c' , and the two sets of rays will be in a condition to interfere, though the greater amplitude of the c vectors will prevent their complete cancellation over the shaded areas.

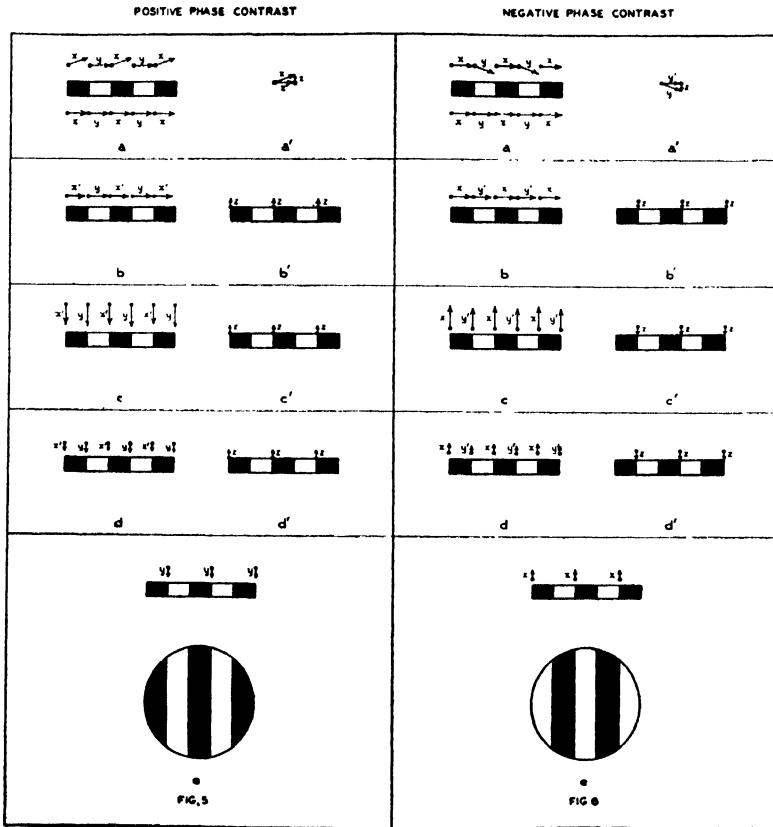
The next stage consists in reducing the amplitude of the c vectors by means of light-absorbing material, and the result will now be as shown in d and d' .

After destructive interference has taken place, the effect will be as at e , and in the final image the areas corresponding to the dense portions of the image will receive no light, while those corresponding to the less dense areas will be evenly illuminated.

With organic preparations the wave-fronts necessarily become very complex,

but contrast is built up point by point over the whole field of view by a redistribution of the luminous energy, and the images produced by phase plates of $+\lambda/4$ and $-\lambda/4$ will appear as positive and negative. In the former case the dark areas correspond to places of higher refractive index in the object, as in fig. 5. Negative phase-contrast is represented in fig. 6.

Two important points emerge from this examination. It is clear that, in order to obtain maximum contrast, the reduction in amplitude of the light



Figs. 5 and 6.

proceeding through the annulus must be related to the phase retardation produced by those parts of the object that it is desired to see in high contrast. Perhaps on a future occasion it will be possible to elaborate on this important aspect of phase-contrast.

It now remains to describe the particular optical arrangements which characterize the microscope brought here for demonstration this evening. It is a standard binocular instrument (fig. 7) converted to phase-contrast by the removal of the normal substage condenser and objectives and the substitution of special parts. These consist of :

- (a) A rotating changer carrying a set of condenser annuli (one for each objective) mounted below a special condenser of 1.0 N.A. Each annulus can be centred by means of two centring screws.

- (b) A set of phase-contrast objectives giving initial magnification of $\times 10$, $\times 20$, $\times 40$, and $\times 95$, each fitted with a phase-retarding annulus in its back focal plane. This phase plate only slightly affects the performance of the objectives when used in a normal manner.
- (c) An auxiliary microscope for examining the back focal plane of the objectives and ensuring that the image of the condenser annulus is in coincidence with the phase plate.

Particulars of the optical arrangements are as follows :

An annular diaphragm (fig. 8) is mounted in the anterior focal plane F of the substage condenser so that the object Z is illuminated by a hollow cone of light. The direct image of this brightly illuminated annulus is formed by the objective O in its back focal plane F', together with other diffraction images (due to structure in the object) which are displaced from the optic axis. In the plane F' is placed the phase-retarding plate, which consists of another annulus exactly matching the direct (or zero order) image of the condenser annulus. This introduces a phase difference of $\lambda/4$ between the rays which are directly transmitted and those diffracted by the object which pass to one side of the phase-plate annulus.

The phase-plate annulus is metallized in order to absorb a proportion of the direct rays and to assist in the correct lining up of the phase plate with the image of the condenser annulus.

ADJUSTMENT.

The microscope is set up in the usual way, except that the special condenser unit (fig. 9) is fitted to the instrument in place of the standard condenser and the phase-contrast objectives are screwed into the objective changer.

The condenser annulus changer (1) is rotated until the figure 0 appears in the circular window, the substage iris is partially closed, and the object is brought into focus, using the required objective, the annulus changer being then rotated until the appropriate magnification figure (10, 20, 40, or 95) appears in the window.

The substage iris is now fully opened and the eyepiece is replaced by the auxiliary microscope (2), which can be adjusted upon unclamping the screws (3 and 4) until the brightly illuminated condenser annulus is sharply focused and can be seen overlapping the grey ring of the phase plate (fig. 10).

The condenser annulus is centred by means of the screws (5 and 6) until its image is concentric with and is completely covered by the phase annulus. If necessary the condenser may be raised or lowered slightly in order to perfect the registration, and a slight movement of the substage mirror may be desirable to ensure that the annulus is uniformly illuminated.

The system is then in adjustment, and on replacing the auxiliary microscope by the eyepiece a phase-contrast image is observed. The registration of the two rings must be reset as above whenever the objective is changed, but it will be found that with practice this becomes a very simple and rapid operation.

If it is required to examine the object under bright-field conditions, it is

only necessary to rotate the annulus changer until the "0" appears in the window and close the iris diaphragm to the desired extent.

The author acknowledges his indebtedness to the Directors of the following institutions for the preparations and permission to publish the photographs which illustrate this paper: National Institute for Medical Research, Strangeways Research Institute, and Laboratories of the Imperial Cancer Research Fund.

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DESCRIPTION OF PLATES.

PLATE I.

Living preparation of sarcoma cells infiltrating muscle fibres by phase-contrast microscopy. Note the different appearances of the muscle striations probably due to the extent to which they are compressed; also the nerve ending (slightly out of focus) on the muscle fibre at the lower left-hand corner of the photograph. (N.I.M.R.)*

PLATE II.

- No. 1.—Living microfilaria from adult worm by transmitted light. Sheath surrounding organisms not visible. (N.I.M.R.)
- No. 2.—The same field as preceding figure by phase-contrast microscopy. Note indications of enveloping sheaths, although flare obliterates their outlines in the vicinity of the organisms. (N.I.M.R.)
- No. 3.—Living microfilaria in blood of monkey by phase-contrast microscopy showing enveloping sheath. (N.I.M.R.)

PLATE III.

- No. 1.—Telophase of primary spermatocyte of locust by phase-contrast microscopy. (A. Hughes and A. Martin.)
- No. 2.—Structure of a bacterium—*Caryophanon latum*—as demonstrated by phase-contrast microscopy. (N.I.M.R.)
- No. 3.—Structure of a binucleate malignant cell (sarcoma) as demonstrated by phase-contrast microscopy. (N.I.M.R.)

PLATE IV.

- No. 1.—*Pleurosigma angulatum* by phase-contrast microscopy. (N.I.M.R.)
- No. 2.—Blood counting chamber. (a) Positive phase-contrast. (b) Negative phase-contrast. (Cooke, Troughton, and Simms.)

PLATE V.

Text Figures Nos. 7 to 10.

* N.I.M.R. refers to National Institute for Medical Research.



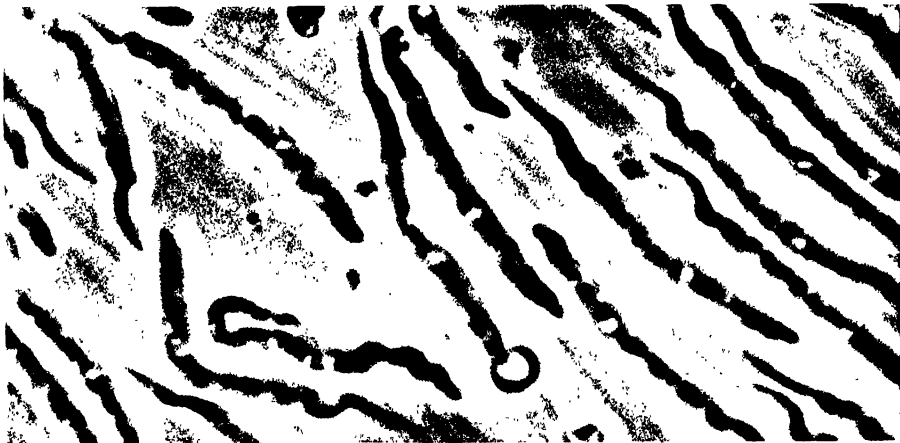
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[To face p. 8.]



No. 1.

810.



No. 2.

810.



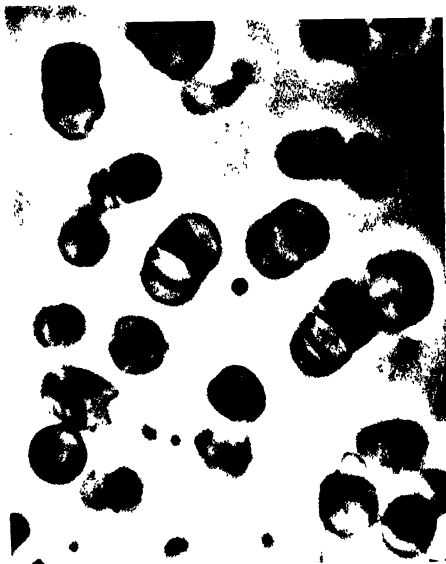
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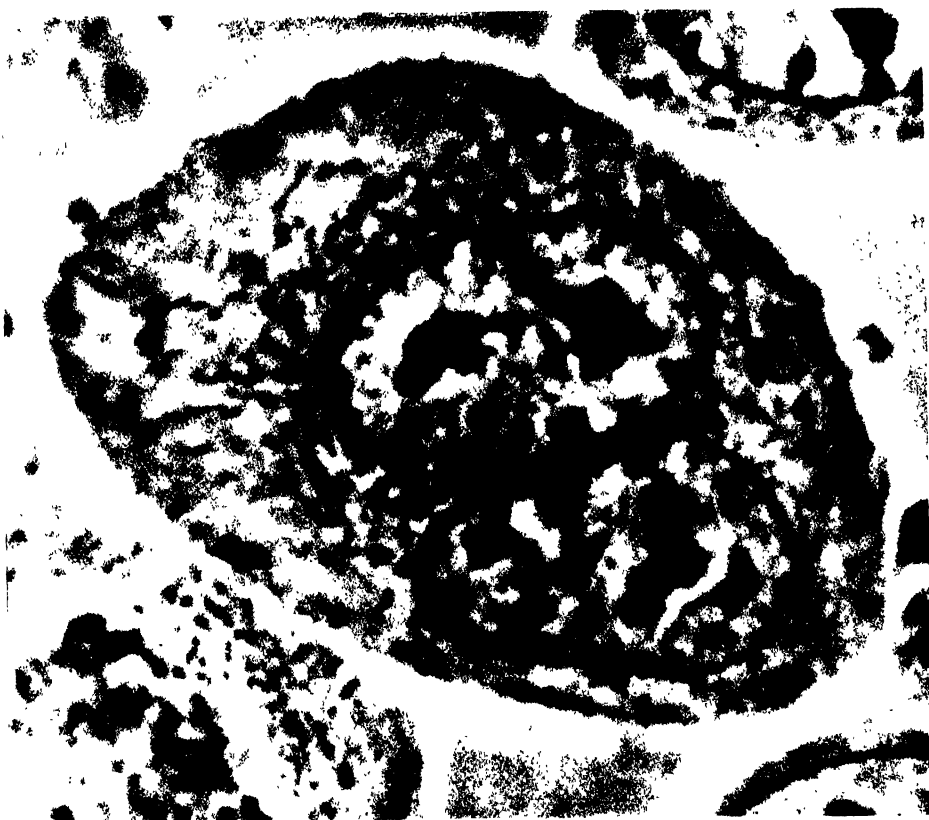
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No. 2.

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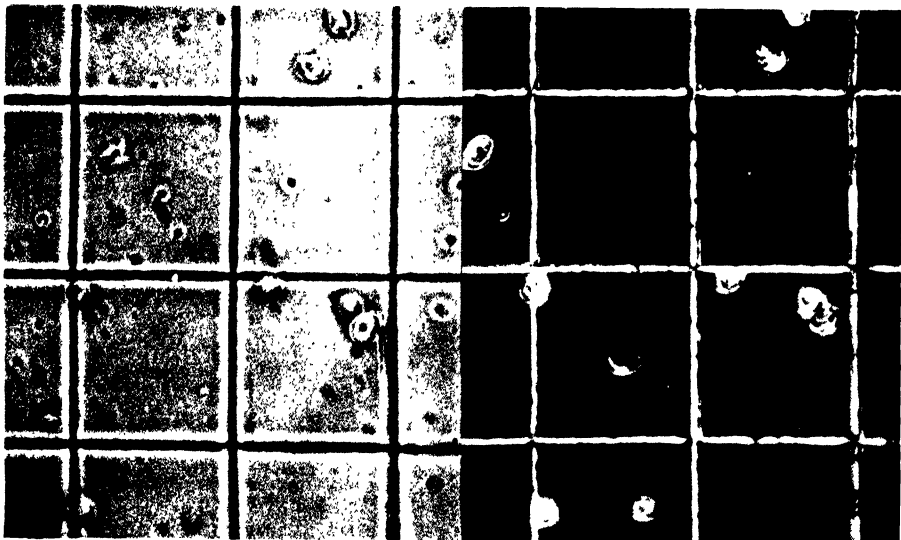


No. 3.

× 2600



No. 1. 1000.



(a) No. 2. (b)

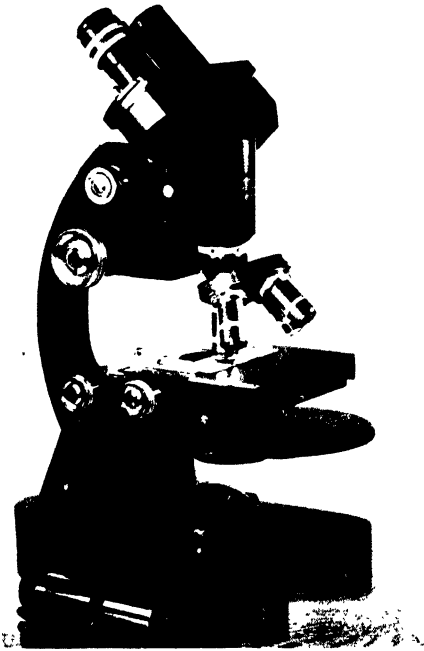


Fig. 7.

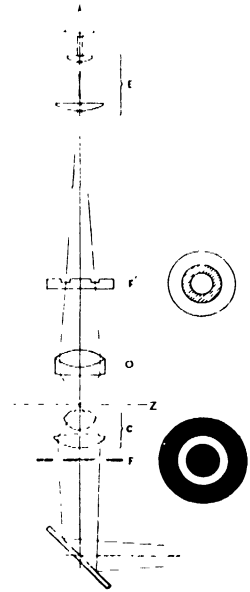


Fig. 8

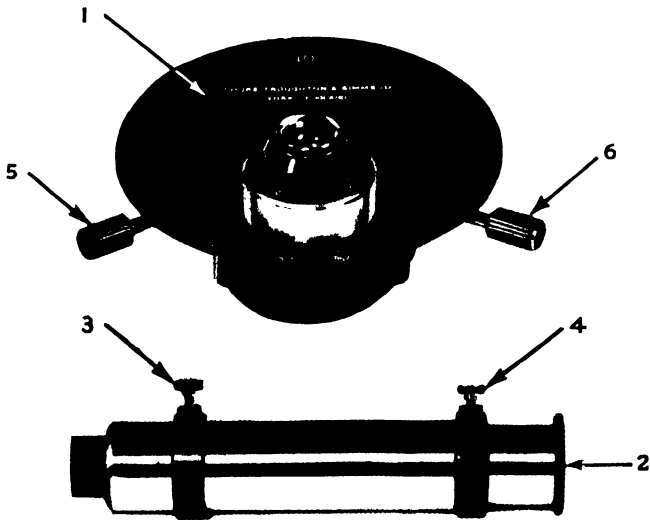


Fig. 9.

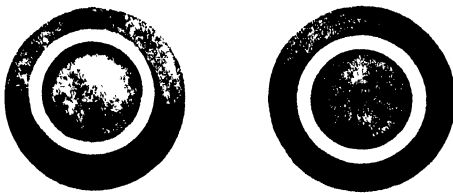


Fig. 10.

II.—THE MICROSCOPIC EXAMINATION OF ENAMELLED WIRE.

By J. H. WREDDEN, F.R.M.S., F.R.S.A.

SIX PLATES.

THE object of undertaking this work was to ascertain the differences in the structure of enamel films commonly used for covering the finer gauges of wire used in the electrical industry.

The covering of these wires with such a film is carried out in order to eliminate the thicker coverings composed of silk or cotton yarn. By using enamel (or varnish) it has been found that adequate interturn insulation is achieved with much thinner coverings with a corresponding improvement in the space factor, which latter assumes a greater importance as coils become smaller and use finer conductors, in consequence of which the number of turns available for any given cross-sectional area of coil is limited, and as is usual in such cases, the designer invariably requires more copper in a particular cross-sectional area than is capable of being furnished by the materials available.

Thus it will be seen that there exists a constant struggle to produce better quality enamels which may be used as thinner films on these wires.

There are, however, many factors tending to limit the thickness of these films ; perhaps the chief of these is the mechanical stresses to which these wires are subjected under actual winding conditions, particularly when these latter are applied to the quantity production of fine wire coils for electro-magnetic devices. The stresses take the form of abrasion of the enamel film, which occurs during the winding (which incidentally is carried out at the highest possible speed commensurate with the physical characteristics of the coil) and in some cases, under operating conditions. Stretching also occurs during winding. This latter effect is frequently unavoidable, as owing to the necessity of packing the winding space with the maximum possible number of turns (which is calculated on the ideal diameter of the wire, and further ; to the fact that seldom if ever is this diameter constant, that is to say, it more frequently errs on the large side than otherwise), somewhat high winding tensions have to be employed in order to wind the coil as tight as possible and in such circumstances it is not infrequently found that whereas the wire on the reel is one gauge, that on the coil may be as much as one gauge smaller, from which the magnitude of the stresses set up in the enamel film may be appreciated to some extent.

The abrasion encountered during the operative life of the coil may be quite simply minimized by impregnating the whole component with a suitable compound. The effects of this abrasion are not regarded as very serious, except perhaps in cases where the turns are relatively loose and the coil operates on alternating current, in which case it will be seen that the loose turns, and in fact the whole coil, is in a state of continual vibration during operating periods,

but this condition seldom occurs owing to the tightness of winding, and the general practice nowadays of impregnating coils with some compound which sets hard and holds the turns rigidly in place.

The abrasion produced during winding cannot, however, be so easily combatted, there would appear to be only two courses capable of adoption in this instance. First, the enamel may be made so strong as to be capable of withstanding the greatest disruptive force liable to be met with, and secondly, the path of the wire may be made as smooth as possible by winding it over free-running pulleys. This latter expedient is, of course, extensively used, but it cannot prevent the abrasive forces experienced by the wire when so-called "random" winding is indulged in, where the rubbing occurs in the coil during winding and the user must therefore rely on the toughness of the enamel film on the wire, allowing him to produce an electrically sound winding by these means.

As can be imagined, there are various types of wire enamels, some better than others, but in general the most popular type up to the outbreak of the Second World War, was based on the use of china-wood oil, which commodity is well known as being almost insurpassable as a paint and varnish medium, and wire enamels using this substance had reached a pitch of perfection where there was little difference, if any, between the products of manufacturers of repute.

The outbreak of war, and consequent disorganization of world supply routes, had an almost immediate effect in this country on the question of supplies of this valuable commodity, which dropped to a fraction of the normal requirements, as a result of which we were forced to start existing on the stocks then available here. However, the commencement of hostilities in the Far East resulted in an almost complete cessation of supplies to this country.

But parallel to this there occurred the vast and rapid expansion of the plastics industry, more particularly in the United States of America, and to some extent in Britain, in consequence of which the development and use of an ever-increasing number of new and otherwise little-used synthetic resins was greatly accelerated. Perhaps the most outstanding of these were the super-polyamide resins more commonly known as Nylon.

This expansion of the use of synthetic resins was fortunate because, as can be expected with the shortage of china-wood oil, the quality of wire enamels began to fall off at a time when it was least desirable, as the electrical equipment made at the time was almost exclusively used for war purposes and required to be of the maximum reliability.

Now whereas before the outbreak of war the wire enamellers had developed, and were still developing, the use of synthetic resins in conjunction with china-wood oil, this development was directed into channels mainly dealing with enamels composed entirely of synthetic materials, which resulted in the production of wire coverings possessing hitherto undreamed-of properties.

These coverings may be regarded as being in the nature of super-coverings, both as regards quality and price, nevertheless they were of great use during the war and, no doubt, as the demand increases and the raw materials situation eases, they will come into more general use.



Fig. 1.



Fig. 2.

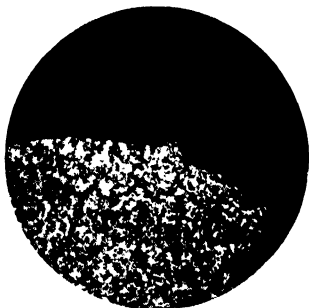


Fig. 3.



Fig. 4.



Fig. 5.



Fig. 6.

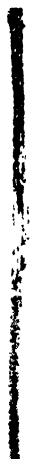


Fig. 7.

On the appearance of samples of these newer coverings, it was decided to test and examine them very thoroughly in conjunction with the standard materials obtainable at the time, and the remainder of this paper is an account of the results and conclusions produced by subjecting them to microscopic examination.

In the first instance a spate of trouble was experienced with the standard wire when used for coils impregnated in a bakelite type A varnish, this latter consisting of a phenolic resin dissolved in alcohol. The cause of failure was suspected as being due to the presence of appreciable quantities of free phenol in the varnish, and on testing the wire by boiling it in alcohol, formalin, and phenol, it was established that the only constituent which affected the enamel film was the phenol, which succeeded in bringing about a complete disintegration of the varnish film in a matter of seconds.

As a result of this, further tests were instituted on the standard film and two of the new enamels, thus at first there were three samples, (A) being the standard enamel, (B) being a synthetic enamel consisting of polyvinyl-acetal, and (C) being a covering of Nylon.

On subjecting these films to the action of phenol, in the manner just described, it was found that their behaviour was somewhat characteristic. Thus in fig. 1, we have a photomacrograph of a sample of the standard wire after having been heated in phenol. As can be seen the enamel film has undergone complete disintegration, leaving the conductor exposed, this shown at *a*. The magnification is approximately 2.5 diameters.

The wire illustrated in fig. 1 is a heavy-gauge material and in order to see if the same effect occurred with wires of smaller diameter, the same test was carried out with a wire of 37 S.W.G. The effect on this wire is shown in fig. 2, this being a photomicrograph of the result, at a magnification of 47 diameters. This illustration clearly shows the same disruptive effect produced by phenol, the enamel has again been disintegrated leaving the conductor bare, so that it did not seem to matter what the gauge of wire, the enamel film suffered in much the same way.

There was one curious effect which called for a certain amount of investigation, this was the manner in which the enamel film broke down. It was noticed that every time this standard enamel was tested in phenol, it broke up by first parting at the end of the wire at two or three points on the periphery. These then developed into longitudinal splits in the enamel coating along the length of the wire, and finally the film broke up into a corresponding number of streamers, some of which started curling at the bottom and finished at the limit of the phenol surface.

Conjecture on this point led to the hypothesis that the splits occurred along lines of strain in the enamel film, these latter existing by virtue of the presence of longitudinal ridges on the surface of the conductor, in consequence of which the enamel film would be strained in this direction.

This point will be made clearer by reference to fig. 3, which is a photomicrograph of a transverse section of some copper wire, showing just such a ridge in section. From this it will be seen that when the enamel film is applied to the outside of the wire, it will pull down on drying so that a thin zone will exist

along the peak of the ridge, which will be comparatively highly stressed so that when the enamel is structurally weakened, such as would occur in the case of the attack by phenol, then the film would tend to rupture along this line, producing the observed effect.

By way of interest, it may be pointed out that these ridges are undesirable, both from the point of view of electrical efficiency, due chiefly to the thinning of the enamel film, and also possible mechanical effects due to adjacent turns in a coil cutting into one another.

Continuing with the examination of the newer synthetic enamels under the same conditions, fig. 4 shows a photomicrograph of some wire (of the same type as B) that is to say the polyvinyl-acetal film, after the phenol test. In this case we have a different effect, albeit complete disintegration of the film has again taken place, but the manner of breaking up is quite different from that exhibited by the previously examined specimen, for it will be noticed that the film breaks down more by flaking than any other way, but this is just as effective in exposing the copper as the method of break-up shown by the standard enamel, but in this case the disintegration of the film took somewhat longer than in the former case.

The effect is the same with this type of film for all gauges of wires, as shown by fig. 5, which is a photomicrograph of some 24 S.W.G. wire covered with this material, at a magnification of 47 diameters. In this case the higher magnification shows clearly how the enamel film disintegrates by curling up into small flakes. The effects of longitudinal ridges are also shown in this photograph. So that taken "by and large" one could say of the polyvinyl-acetal film, that it is only slightly more resistant to phenol than the standard enamel. This extra resistance, however, is so small to be negligible for all practical purposes.

In actual fact the resistance to phenol of all these wire coverings is low; as can be imagined, even Nylon, which is considered to be a highly resistant substance, shows considerable weakening under the same conditions. This is well shown in fig. 6, this being a photograph of a Nylon-covered wire after the same treatment. Here again it will be seen that an effect different from the two previous samples has been produced. The film shows itself to be considerably more resistant to this test than the others, inasmuch as it does not appear to break up into relatively small pieces, rather does the film as a whole undergo considerable swelling, retaining its tubular form. The crinkling of the film is due to the fact that it swells in all directions, that is to say, it increases in diameter and at the same time elongates and slips off the end of the wire. In the sample shown, the cut end of the conductor is rough and has prevented the Nylon film from sliding freely, with the result that the expansion is taken up by the tube folding on itself as shown.

Although when in this condition the Nylon film is very delicate, it does by virtue of retaining its original form, offer a greater resistance to attack by phenol than the previously discussed coverings, and indeed this covering has shown itself to be superior in all manner of drastic physical and chemical tests imposed upon these enamelled wires.

A fourth sample which was stated to be different from any of the others was also tested, and here again a characteristic reaction to the phenol test was obtained. This is shown at a magnification of 4 diameters in fig. 7. It will

be seen from this illustration that the covering has, in general, shown signs of crinkling, with the exception of one patch in the centre where the conductor has been bared for a short distance.

The behaviour of this specimen during the test was extremely interesting, inasmuch as that initially the entire covering exhibited the swelling in all directions, which was characteristic of the Nylon film, but after a matter of fifteen seconds or so the covering was seen to develop an uneven surface and crumpled up quite suddenly, when the central patch appeared to burst open.

Bearing in mind the behaviour of the standard enamel which disintegrated suddenly and appeared to burst open, and later the Nylon covering, it seemed very much as if this new sample was covered with something of a dual nature ; that is to say, its behaviour would lead one to suspect that a coating of similar nature to Nylon has been applied on top of one akin to the standard material, for such a material would behave in a manner similar to that observed.

A better idea of this type of disintegration is obtained by reference to fig. 8, which shows this, presumably double-coated wire, at a magnification of 47 diameters, and in which the disruptive effect of the under coating is shown. It will be seen that this has been strong enough in this case to actually break through the outer coating.

In order to check this hypothesis regarding the duplex nature of the coating, it was decided to endeavour to produce a section of the wire so as to make this visible, if it existed, and after much experimental work, which will be discussed subsequently, this was accomplished with the result shown in fig. 9. This is a photomicrograph of a longitudinal section of this wire at 460 diameters and in which we see at *a* an outer transparent film, while at *b* is an inner coating of a dense black or nearly black colour, at *c* we see the copper surface, the ragged edge of which is due to light being reflected off the crystal faces of the copper. In this manner then, the existence of a suspected double coating was proved conclusively and further inquiry elicited the information that the under coating was more or less a standard grade of enamel, while the outer covering was composed of Nylon.

After having obtained this preliminary information, it was decided to carry the microscopic examination further, in order to obtain as much information as possible about these wires. Therefore the specimens were examined both in the normal state and after being subjected to various treatments. These were as follows :

- (a) Examination of the normal covering as received.
- (b) Examination of the effect on the covering of a 10 p.c. elongation of the wire, by stretching.
- (c) Examination of the behaviour of the covering at the point of fracture brought about by a steady pull along the sample.
- (d) Examination of the effect on the covering of bending the wire round its own diameter without having previously been stretched.
- (e) Examination of the effect on the covering when treated as for (d) but after having been heated in an oven, at a temperature of 150° C. for 4 hours.

From the foregoing remarks it will be appreciated that the tests imposed on the samples were drastic to say the least of it, and therefore any which is capable of standing up to this treatment must be somewhat unusual.

It is proposed, for the purpose of this test, to examine the effects produced on the four samples already discussed, they are :

- (1) Ordinary or standard enamel.
- (2) The polyvinyl-acetal covering.
- (3) The Nylon covering.
- (4) The double covering consisting of an inner layer of standard enamel covered with an outer layer of Nylon.

It will be seen that we have five groups of four results and in examining the results produced by the examination of the normal film, we see in fig. 10 the normal enamel at a magnification of 47 diameters. It will be noticed with this film that there is very little visible structure, but this is to be expected from the nature of the enamel and the lighting conditions under which the photograph was taken. Such of the structure as is visible appears to consist of two types, one being a coarse unevenly distributed granular structure which is only faintly portrayed, the other being a very much coarser structure showing in the illustration as comparatively large areas of light, and in which can be detected short transverse lines. As this enamel is, of course, of a colloidal nature, it is probable that these large light areas represent agglomerates of colloidal particles, however, they do make their appearance at fairly regular intervals along the length of the wire, and may be taken as being more or less characteristic of this type of enamel, although it must be borne in mind that the same varnish applied by different methods will modify this tendency to produce large colloidal agglomerates. Five of these patches are annotated at *a*, *b*, *c*, *d*, and *e* in fig. 10.

With regard to the faint transverse lines observed in this coating, it would appear that they exist by virtue of stresses set up in the film, caused by shrinkage taking place during the processing. Their visibility is probably due to the oblique lighting taking effect at the junction plane of two slightly different refractive indices, these latter occurring in the film at these points of stress. If this hypothesis has any foundation in fact, it will be clear that these stressed points are potential areas of weakness in the film, which on being subjected to a longitudinal strain, may be expected to develop into actual cracks in the enamel film, and in view of the stressing undergone by the wire during winding, it will be appreciated that weakness of this kind, if present to any great extent, is highly undesirable.

However, the tenability of this theory may quite easily be assessed by examining the same coating after treatment according to test (*b*) that is to say, after having been stretched 10 p.c. of its original length. The result of this is shown in fig. 11, which shows this wire thus treated, at a magnification of 47 diameters. This shows the development of further transverse marks and also the presence of small transverse cracks at *a*, *b*, *c*, and *d*, the existence of which lends some measure of support to the aforementioned hypothesis.

As a result of this effect, one would be led to the assumption that the film lacks elasticity, which characteristic is, of course, eminently desirable owing to



Fig. 8.

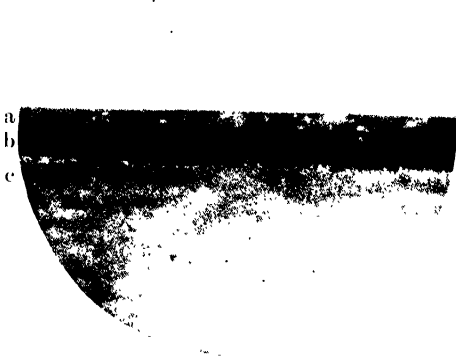


Fig. 9.

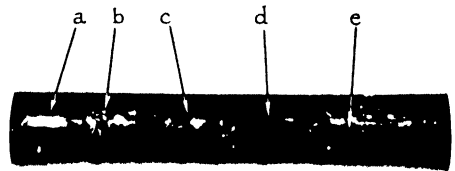


Fig. 10.

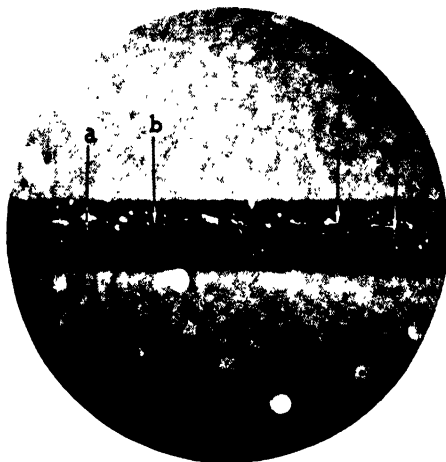


Fig. 11.

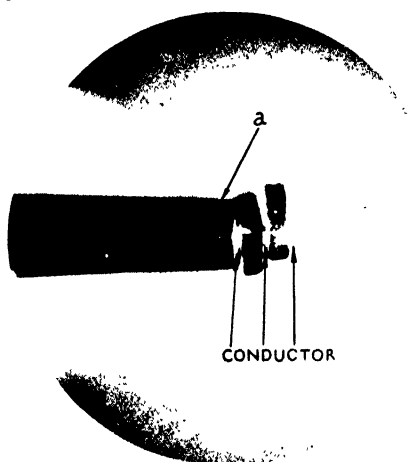


Fig. 12.



Fig. 13.



Fig. 14.



Fig. 15.

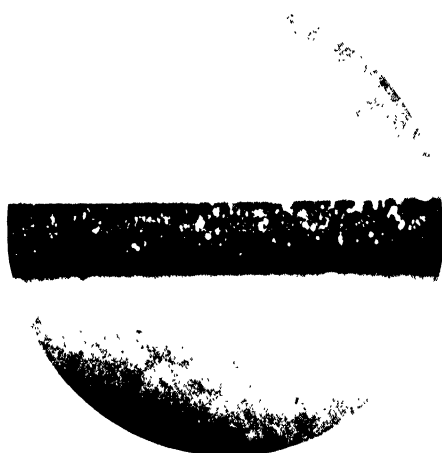


Fig. 16.

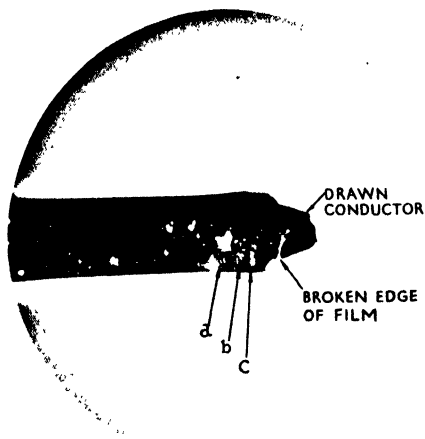


Fig. 17.

[To face p. 15.]

the nature of the duties required of it. Thus with an inelastic film, the presence of stress marks may be expected rather than otherwise, as any stresses set up in the film during application would tend to remain more or less *in situ* and not absorbed as would be the case in an elastic body.

The relative inelastic quality of this type of enamel is well shown in fig. 12 which illustrates the result of test (c) (i.e. extension to fracture) at a magnification of 47 diameters. The fracture of the enamel film will be seen to have occurred as a spiral with clean edges. This specimen is of interest because actually the fracture existed as just one straight edge, but while watching the specimen under the microscope the spiral split was seen to develop, taking a matter of half a minute or so to complete. This phenomenon obviously occurred as a result of the release of stored energy in the film due to its inelastic nature, and occurrences such as this would appear to be characteristic of such films. It should be borne in mind that although this enamel is seen to be inelastic under these tests, it is in reality quite good when compared to ordinary enamels. Its weakness in this property should, therefore, be regarded as being relative to the films shortly coming under discussion.

In continuing the examination of this wire, it is now proposed to look at the effect produced on it by test (d). This test involves the bending of the wire round its own diameter without any previous treatment, and the effect produced by it on the standard enamel is shown in fig. 13, again at 47 diameters, which shows how the enamel film splits on the outside of the bend and "ruckles" up on the inside. Although the breaking up of the enamel in this instance appears to be severe, it should be remembered that the test itself is very severe and in these circumstances the enamel may be considered to have stood up to the treatment quite well.

However, when we come to examine the effects produced by test (e) we are confronted by a rather more serious condition, as this test may be considered to be in the nature of a very greatly accelerated heat ageing, and as can be seen from fig. 14 the standard enamel has reached the limit of its endurance, as it were. It is not by any means suggested that a test such as this should be regarded as standard, and this type of enamel was subjected to this treatment more as a means of producing a working standard on which to compare the results of the test on the more highly resistant film. In actual practice the standard enamel, and for that matter the newer synthetics, are seldom, if ever, subjected to treatment such as this and therefore this test should be looked upon only as a means of obtaining comparative information as to the behaviour of these coverings.

Having examined the effects of these tests on the standard enamel, it is now proposed to do likewise for the other samples, the next on the list being the polyvinyl-acetal covering. Thus in fig. 15 we see the normal covering at a magnification of 47 diameters. This illustration shows a big difference in structure from the standard material, inasmuch as it does not exhibit the heavy agglomerates of colloidal particles seen in the previous case. The film is of course colloidal and as such the presence of the small, more or less evenly distributed particles is not unexpected. A further interesting point is brought to light in this photograph by the arrangement of these particles which tend to

place themselves in longitudinal rows. This phenomenon is interesting as the film is composed of a synthetic resin, the molecular structure of which is based on chains of great length and as is well known, if under these conditions, the molecules can be persuaded to arrange themselves in rows parallel to one another, then the mechanical strength of the material is considerably increased as a result of this molecular orientation.

As the coverings of these wires are applied in general in the form of a more or less viscous solution, with the wire passing through a circular die, it will be seen how the film would be subjected to an action somewhat akin to drawing, as a result of which there would in all probability be a distinct tendency to "comb out" these long molecules so that they would lie longitudinally, particularly as these synthetic resins are applied at high viscosities. This does not, of course, mean to say that every material of this type undergoes enormous increase of strength by bringing about molecular orientation, it is obvious that the length of the chain and the degree of orientation will have a great influence on the final properties of the material; thus in this case we may say that, whereas there are signs of molecular orientation present, this would appear to amount to a little more than a tendency towards this condition.

That this is so is well shown by reference to fig. 16, which shows the same film after having been stretched for 10 p.c. of its length. This illustration, which is at a magnification of 47 diameters, shows how the stretching breaks up what little regularity there was originally in the structure, for the particles are now seen to be more unevenly spaced and the whole structure becomes somewhat coarser than that exhibited by the untouched covering, from which it will be seen that a depreciation of mechanical strength may be expected.

The effects of molecular orientation are, of course, reflected in various properties, one of these being the type of fracture when the materials are stressed to this point. It can be shown that where the orientation is high the fractured edge assumes a ragged condition, and in cases where there is little or no orientation the fractured edge is straight and clean. This we saw was the case when examining the fracture of the standard material. Also, a highly orientated material could be expected to possess a greater degree of inherent elasticity than the unorientated. In fig. 17 we see the fracture produced by the polyvinyl-acetal covering, where the broken edge of the film is seen to be somewhat ragged, although there are straight portions. These latter indicate the existence of only partial orientation, which if complete would have produced a more ragged break, owing to the position of the stress line at the time of the fracture being dictated by the alignment or otherwise of the ends of the molecular chains.

Bearing in mind the foregoing remarks regarding the nature of this film, let us examine the effect of bending the wire round its own diameter. This is shown in fig. 18 at a magnification of 47 diameters, from which it will be seen that this covering is a big improvement on the standard enamel, although the usual effect of stretching, in the form of the granular structure at *g* will be noticed, also small cracks at the points marked *a*, *b*, *c*, and *d* may be seen to have made their appearance, as also has the ruckling on the inside of the bend at *e*, but taken on the whole this covering represents a distinct advance on the one previously discussed.

The greater resistance of this covering is further illustrated in its behaviour under test, which involves the heating for 4 hours at 150° C. The result of this is shown in fig. 19 where the most pronounced effect is seen to be in the development of a coarser structure in the film and the appearance of larger cracks at *a* and *b*, but here again the covering has withstood the rigid test conditions remarkably well, when compared to the behaviour of the standard enamel.

So much then for the polyvinyl-acetal covering, now suppose we proceed to the examination of the Nylon covering treated in the same way. The normal film is shown in fig. 20, and the most notable point of this is the evidence of strong orientation as indicated by the fine striated appearance. This would appear to be a visual indication of the effects of orientation, because obviously, whereas it is an impossibility to see the actual molecules themselves, it is reasonable to suppose that the order of their arrangement might become visible under a cumulative effect in much the same way as the shape of a pure crystal is in some measure an indication of its lattice nucleus.

If we now examine the effect of stretching as shown in fig. 21, again at 47 diameters, it will be seen that the striated structure has more or less disappeared. This is due to the fact that as the film is stretched in the direction of molecular orientation, this would tend to become tighter; in other words, if in the normal film the lines of molecules are a certain distance apart, longitudinally, then on stretching the film in this direction, these gaps would close up and instead of the material becoming structurally coarser, as we saw occurred with the two previous specimens, it actually becomes finer and within certain limits it also becomes stronger, because as a little thought will show, the film in this case has actually undergone a process analogous to cold drawing. So that here we have a reversal of the behaviour of the other two films which is on the credit side and points the way to what might be expected of the Nylon film in further tests.

The high degree of orientation existing in this film is confirmed by the fracture as shown in fig. 22, where the edge will be seen to be ragged and containing less straight portions than the polyvinyl-acetal film. It will be seen from the examination of these fractures that each of these materials shows a characteristic edge. It is not suggested that the method may be used for identifying any particular films, but rather should it be used as a means of confirming previous deductions, such as the presence or otherwise of molecular orientation in the covering, etc., so that it will be seen to be more in the nature of a group test by means of which the material may be placed in one of a number of groups, according to its behaviour under working stress.

We have seen that due to the high degree of orientation in the Nylon covering, its general behaviour to the tests so far applied is somewhat different from that of either of the two previously examined samples, the result showing the Nylon to be superior. Therefore, one would expect this covering to behave well under the bending test as described for (*d*), particularly in view of the tightening up of the orientation on stretching. That this assumption is justified, is well shown in fig. 23, which illustrates the result of applying this test to the Nylon covering, and from which it will be seen that little if any damage has resulted from bending this wire round its own diameter. There are no cracks or signs of any appearing and no sign of any ruckling to be seen on the inside of the bend.

This result, however, is to be expected rather than otherwise, but the most remarkable result is shown by the behaviour of this covering under the conditions of test (e), which is shown in fig. 24, again at a magnification of 47 diameters. From this illustration we see that in spite of being stoved for 4 hours at 150° C. the Nylon covering remains perfect under the severe conditions imposed on it in the bending after this heating.

The next wire to come under consideration is that possessing the double covering. It has been demonstrated that this covering consists of an inner coating of ordinary enamel covered by a film of Nylon, and from the previous behaviour of this material a similar type of intermediate result may be expected under the new test conditions.

Thus the untouched film as shown in fig. 25, shows the visible effects of orientation in the Nylon film and also here and there, portions of the agglomerates in the standard undercoating. From this it may be assumed that this latter will behave in the same way as it did in the case of the first sample, but that this would be modified and somewhat held in check by the presence of the Nylon coating which will function as a restraining medium.

That this is to some extent true is shown in fig. 26 which shows the familiar closing up of the Nylon structure, but also the minute cracks in the standard film underneath are again in evidence at the points *a*, *b*, and *c*. The nett result of this is to create weak spots at the points indicated both electrically and mechanically, which may be liable to produce disastrous results in service. In general the illustration affords some measure of support to the previously mentioned assumption. The specimen in this case exhibits, of course, the effects produced in this double coating, stretching the wire 10 p.c. of its length as in test (b).

Further evidence of the restraining action of the Nylon coating is given by test (c), dealing with elongation to fracture, the result of carrying out this test on the composite covering is shown in fig. 27, where the drawn-down end of the conductor is clearly visible, as also is the method of break-up of the undercoat, which has split spirally in the same manner as before, but in this case the breaking up of the enamel can be seen to have been held in check by the outer covering. At the same time it will be noticed that the effect produced on the orientation of the Nylon is very clearly shown by the orderly arrangement of the granular structure either side of the crack in the undercoat. The reason for the appearance of this structure in this manner is not exactly clear, therefore, the hypothesis that it is a visible effect produced by the molecular orientation in the Nylon should be regarded as being more of the nature of an assumption than a fact proved by experiment and it is obvious that a great deal more investigation on this point is necessary before definite statements may be made.

When it comes to the bending tests the restraining action of the outer covering comes into play in a remarkable manner, for judging by the effects in previous tests one would not expect the Nylon film to be capable of checking the breaking up of the undercoating in the manner shown in fig. 28, which illustrates this double-covered wire after having been bent round its own diameter and from which it is seen that apart from a small amount of granulation of the undercoating, there are no signs of breaking up and in general the covering as

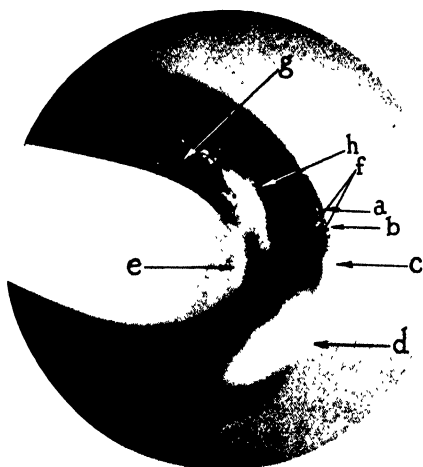


Fig. 18.

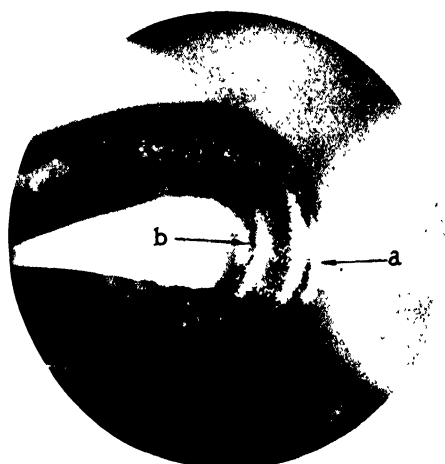


Fig. 19.



Fig. 20.



Fig. 21.

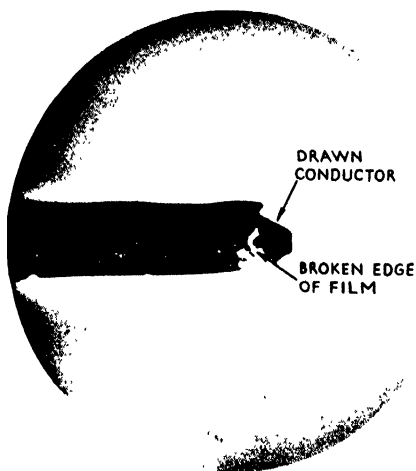


Fig. 22.



Fig. 23

[To face p. 18.]



Fig. 24.



Fig. 25.

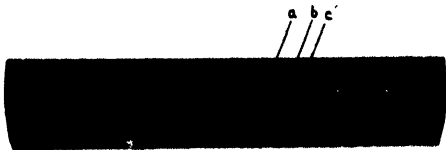


Fig. 26.

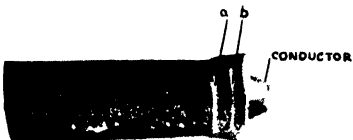


Fig. 27.

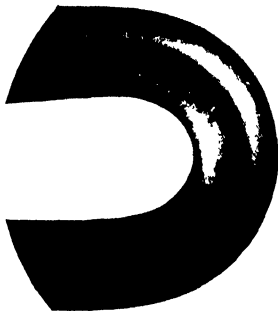


Fig. 28.

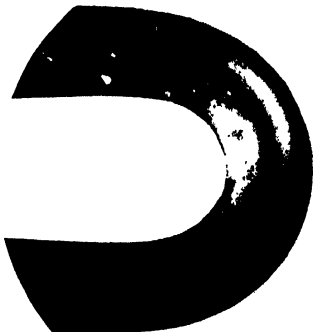


Fig. 29.

a whole may be considered to have given almost as good an account of itself as the pure Nylon covering.

Especially is this so when the effect of bending this wire round its own diameter after heating is examined. The result of this test is shown in fig. 29 which illustrates the somewhat greater degree of granulation which takes place, nevertheless the covering as a whole has remained intact, no signs of any crack being apparent. It becomes clear that by examining these wires under the microscope, much information of value was obtained which would not have come to light otherwise, and at the same time it has been possible to forecast the behaviour of any one of these four types of covering under conditions involving the action of abrasive focus, more particularly is this so when it comes to considering the use of this wire at elevated temperatures, in any event the majority of coils are impregnated with a varnish of some kind, or a wax, which involves the use of high temperatures for quite considerable periods followed by comparatively rapid cooling and as a result of the consequent contraction and expansion considerable abrasion takes place between turns, sometimes leading to serious results if the enamel is incapable of withstanding these forces, particularly if it has been affected by any of the constituents of the impregnating medium such as has already been described.

The methods adopted for preparing the specimens are interesting inasmuch as they occasioned the development of somewhat unorthodox steps to obtain the results produced. Therefore a brief résumé of processing methods will not come amiss.

To obtain the photographs illustrating the effects of phenol on these coverings, it was necessary to photograph them *in situ* as it were. The method adopted was to heat a specimen of the wire in a test tube containing phenol liquor which was rapidly cooled as soon as the enamel showed signs of breaking up. This latter step was necessary in order that the rapid break-up of the enamel may be checked, for it was found that if the boiling was continued for any length of time the enamel was completely broken up and lay in the bottom of the test tube in the form of fine dust, leaving the copper quite bare. Therefore the process has to be stopped, or slowed down, at a point well before finality so that an idea of the mechanism of breaking up could be obtained.

After checking this action the specimen is in a very delicate condition, the slightest jarring being sufficient to dislodge the disrupted enamel film, as a result of which the specimen has to be handled very carefully and photographed as soon as possible, as the action continues slowly in the cold state. The photographs were all taken with a Zeiss Tessar $f3.5$ lens of 75 mm. focal length, stopped down to $f8$, the lighting being straightforward transmitted light. The negative material was Ilford Selochrome in half-plate form.

Apart from the necessity for not disturbing the specimen in any way, there is little that is unorthodox in obtaining these photographs, but the apparatus used may be of interest; it is, therefore, illustrated in fig. 30. As can be seen it consists of an ordinary half-plate, double-extension camera, rigidly mounted at an angle of 45° . The stays for clamping the front were found to be necessary at this latter was very unsteady at long extensions, likewise the specimen stage

was fitted with the rack and pinion motion shown in order to facilitate focusing at long extensions.

This apparatus was designed and produced in this form as lack of space precluded the use of any of the more orthodox types, it has, however, proved to be easy to set up and use, as the image on the screen can be seen during focusing without any trouble, the control being quite conveniently at hand. As the whole instrument is mounted on a solid slate base, it is easily packed away when not in use, thus making room for other purposes.

The photography of the results of the other tests presented a rather different problem, as the microscope had to be brought into use. Thus with the exception of figs. 3 and 9, they were all taken with a $1\frac{1}{2}$ -inch "Parachromatic" objective by Watson, in conjunction with a $\times 7$ Huygenian eyepiece. The tube length employed was 160 mm. with a screen distance of 10 inches.

Initially these specimens were mounted dry being merely held at one end in a piece of plasticine so that the other end is free in air about $\frac{1}{8}$ inch above the surface of an opaque white slide. The lighting was arranged from the top at an angle of approximately 45° , which angle was found to be the best by trial; the specimens were lit from two sides to avoid dense shadows. This method of illumination was adopted for figs. 10-24, from which it will be noticed that the background is not really satisfactory and could do with improvement. It was, therefore, decided to experiment with a view to improving this, the best results being obtained by the use of the top lighting, as previously arranged, in conjunction with transmitted light for supplying the background. Some difficulty was experienced in using this latter, which was eventually overcome by the use of a Chance-Watson yellow-green filter. This enabled the darker tone of the wire to be more nearly fully exposed without over-exposing the background and thus causing troublesome halation at the edges of the desired image. It was further found necessary to mount the specimens in a light mineral oil to avoid halation in the image itself, and also to increase the resolution somewhat. The results of this technique are shown in figs. 25-29.

The section shown in fig. 3 was photographed by quite conventional methods using a vertical illuminator, the specimen itself was moulded into a block of methyl-methacrylate ("Perspex") in the manner common to metallurgical technique to-day.

In the case of the section shown at fig. 9, however, a rather different mounting technique had to be adopted, as it was not possible to mould the wire into any material such as methyl-methacrylate used for the previous section, because the heat and pressure involved tended to disrupt and distort the enamel films.

Other means had, therefore, to be found whereby the wire and its covering could be held securely in place while the sectioning was carried out, and after numerous experiments a method was evolved using methyl-methacrylate resin in the form of "Diakon" granules from which a heavy viscous material was made by the addition of a small quantity of monomeric methyl-methacrylate, this hardened into a white translucent mass, containing large numbers of very small bubbles, when warmed at 60°C . for some 3 or 4 hours.

If the wire is held in position in a crucible, and this material poured round it, the whole can be easily extracted after hardening, leaving the wire firmly

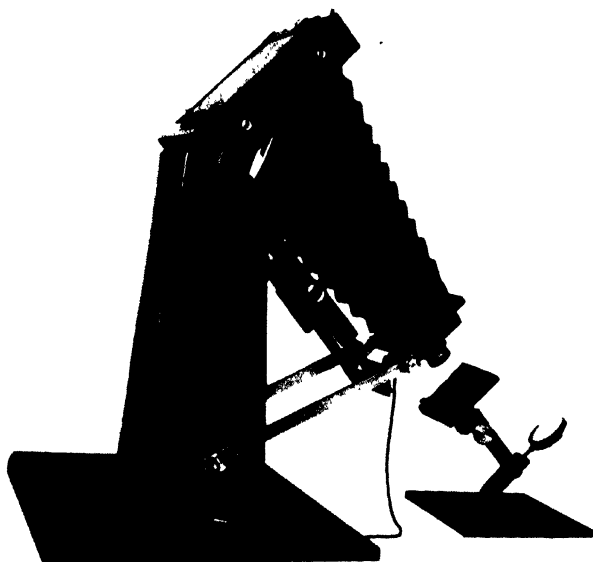


Fig. 30.

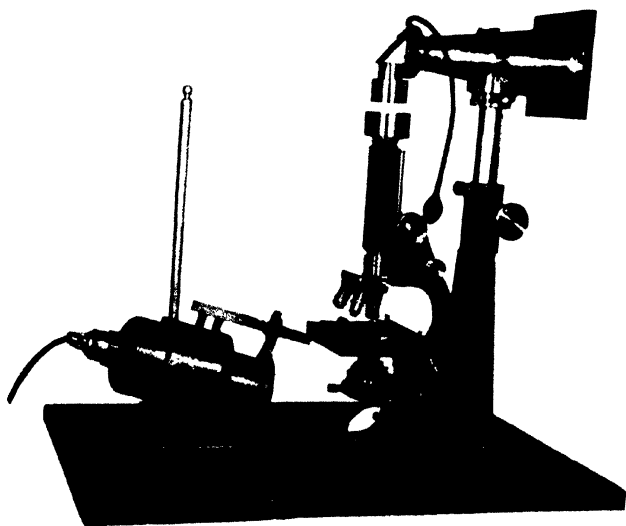


Fig. 31.

[To face p. 20.]

embedded in the solid resinous mass, the subsequent treatment of which follows standard metallurgical practice involving grinding and polishing.

It was at first endeavoured to produce the mass as a clear material, but it was soon discovered that stoving at elevated pressures were required, in consequence of which this idea was abandoned; however, the semi-opaque mass serves the purpose quite well as can be seen from the illustration. The curious point regarding this is the lighting, for it was found that when viewed by means of a vertical illuminator alone, little, if any, signs of the enamel covering was evident and it was only by projecting an intense beam of light through the mass at right-angles to the optic axis of the microscope, that the enamel film was brought up as shown, from which it will be apparent that a transparent or translucent embedding mass is essential in such cases.

This film was photographed with a Watson "Parachromatic" $\frac{1}{4}$ -inch objective used in conjunction with a $\times 10$ Huygenian eyepiece on Ilford's "Selochrome" emulsion, with a screen distance of 10 inches, with the apparatus shown in fig. 31. The tube length was adjusted to give the magnification stated.

Here again the photomicrographic apparatus was designed and made with a view to economizing space, resulting in an instrument of compact dimensions so arranged that while sitting and viewing the image on the screen, the controls are very little displaced from their relative positions as for visual work. As a consequence of this arrangement it is possible to easily obtain a truly critical image on the screen, the effects of such adjustments as focusing the lamp condenser diaphragm and manipulation of tube length, etc., being directly observed on the screen. At the same time a high-powered focusing magnifier may be brought into play on a clear screen, thus making certain of the focus of the image.

The lamp consists of an ordinary "Photoflood" type bulb mounted in the housing shown and working through a Watson-Conrady condenser, fitted with a field diaphragm. The lamp itself is controlled through a resistance which enables it to be used economically and prolongs its life considerably. It has been found to be quite sufficient for photomicrography up to the highest powers, except perhaps where moving objects requiring very short exposures are being photographed.

In concluding this paper, the Author would like to express his thanks and appreciation to Igran Electric Co., Ltd., for permission to publish these results, and for the use of the facilities of the Works Laboratory for carrying out the work. All the illustrations appear by courtesy of the Company.

The Author would also like to thank Mr. R. K. Fleming and Mr. J. Russell Taylor for their helpful suggestions and the permission to reproduce the illustrations as lantern slides.

Thanks are also due to Messrs. Connolly's, Ltd., B.I. & Calenders Cables, Ltd., and London Electric Wire Co., Ltd., who supplied samples and offered helpful advice during the course of the work.

DESCRIPTION OF PLATES.

PLATE I.

Figs. 1, 2, 4, 5, 6, and 7.—Various types of stripping in phenol.
Fig. 3.—Transverse section of copper wire showing die mark.

PLATE II.

Fig. 8.—Enamel stripping in phenol.
Fig. 9.—Longitudinal section showing duplex nature of the coating.
Fig. 10.—Standard enamel untreated.
Fig. 11.—Standard enamel after stretching.

PLATE III.

Fig. 12.—Standard enamel. Point of fracture.
Fig. 13.—Standard enamel after test (*d*).
Fig. 14.—Standard enamel after test (*e*).
Fig. 15.—Enamel No. 2. Test (*a*).
Fig. 16.—Enamel No. 2. Test (*b*).
Fig. 17.—Enamel No. 2. Test (*c*).

PLATE IV.

Fig. 18.—Enamel No. 2. Test (*d*).
Fig. 19.—Enamel No. 2. Test (*e*).
Fig. 20.—Enamel No. 3. Test (*a*).
Fig. 21.—Enamel No. 3. Test (*b*).
Fig. 22.—Enamel No. 3. Test (*c*).
Fig. 23.—Enamel No. 3. Test (*d*).

PLATE V.

Fig. 24.—Enamel No. 3. Test (*e*).
Fig. 25.—Enamel No. 4. Test (*a*).
Fig. 26.—Enamel No. 4. Test (*b*).
Fig. 27.—Enamel No. 4. Test (*c*).
Fig. 28.—Enamel No. 4. Test (*d*).
Fig. 29.—Enamel No. 4. Test (*e*).

PLATE VI.

Fig. 30.—Photomacrographic camera used in tests.
Fig. 31.—Photomicrographic camera used in tests.

III.—GONADAL INVERSION IN THE EGYPTIAN TOAD, *BUFO REGULARIS*.

By M. H. HAIBA, M.V.Sc.

(School of Veterinary Medicine, Fouad I University, Cairo, Egypt.)

TWO PLATES.

THE familiar Egyptian frog, *Bufo regularis*, so far as the author is aware, has not yet been investigated for gonadal inversion. A case of this kind came under the author's notice on April 23rd, 1947, in a male frog which was being dissected for class-teaching purposes.

Macroscopically (fig. 1) the left gonad (A) showed the normal appearance of a testis though it seemed to be slightly enlarged.

The right gonad (B) differed greatly in appearance, the whole gland presenting a fimbriated structure, with cross or radial striations. In total bulk it was about equal in size to the left gonad, but it gave the impression of resembling an immature ovary rather than a testis. No trace of an oviduct, however, could be seen, but a small atrophied urogenital duct could be dissected out. As is well known, the ovary in the frog is not compact, but consists of a fold of peritoneum within which are developed the Graafian follicles. In the present case, that same general appearance was also seen, the whole gland being semi-transparent and punctuated by darker dots.

Histologically, the left gonad presented the normal appearance of a testis (fig. 2), with well-developed seminiferous tubules (A) filled with young and mature spermatozoa (B).

The right gonad, on longitudinal section, showed posteriorly under the low power (fig. 3A) a mass of highly constricted, undeveloped or atrophied seminiferous tubules, which under a higher power (fig. 4) each presented solid colloid undifferentiated contents, in striking contrast to the histological appearance presented by the left gonad (fig. 2, taken under the same high-power magnification).

Anteriorly to the above seminiferous mass (fig. 3C), and also growing inwards from the germinal layer on the surface of the gland (fig. 5A) there was evident inversion into ovarian tissue, with the formation of both large and small Graafian follicles. Under a higher power, the ovulation proceeding within these follicles could be clearly seen.

To ascertain whether or not inversion as described above is common in the Egyptian frog, over 350 specimens were dissected afterwards under water, but in no case was any sign of gonadal inversion observed.

Gonadal inversion in *Bufo regularis*, though it has been here proved to occur, must therefore be looked upon as a rarity.

DESCRIPTION OF PLATES.

PLATE I.

Fig. 1.—Naked eye appearance of gonads *in situ*.

Fig. 2.—Left gonad showing well-developed seminiferous tubules. $\times 450$.

Fig. 3.—Right gonad showing constricted, undeveloped or atrophied tubules. $\times 450$.

PLATE II.

Fig. 4.—Right gonad : colloidal undifferentiated material within tubular spaces. $\times 800$.

Fig. 5.—Right gonad : inversion into ovarian tissue with Graafian follicles. $\times 800$.



Fig. 1.

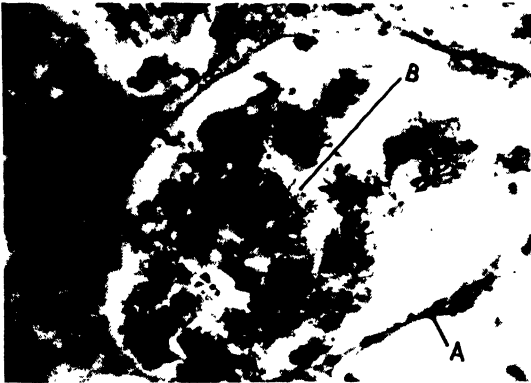


Fig. 2.



Fig. 3.

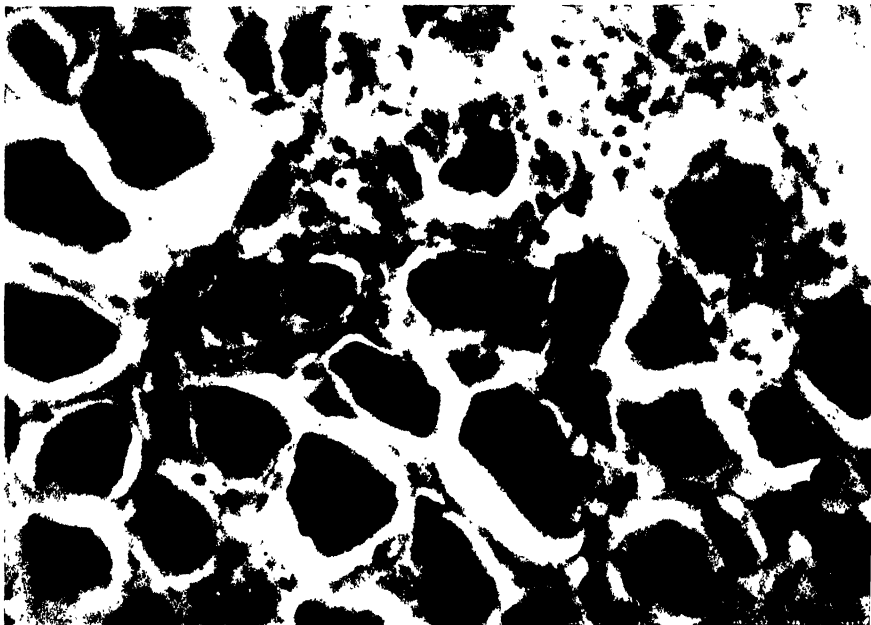


Fig. 4.

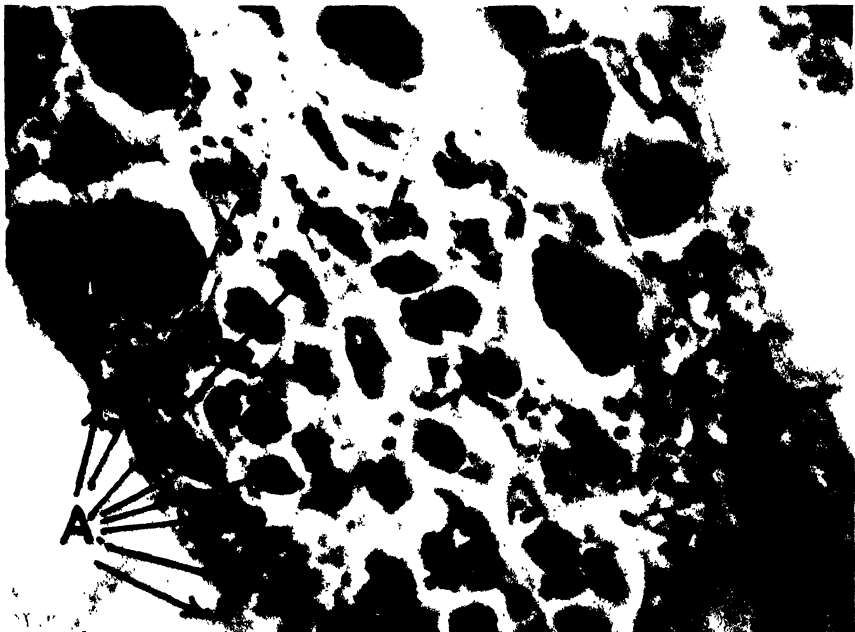


Fig. 5.

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IV.—THE CHROMOSOME SMEAR TECHNIQUE: A CRITICAL REVIEW AND IMPROVEMENT OF METHOD.

By FREDERICK C. GRIGG, F.R.M.S., F.L.S.

TWO PLATES.

1. THE NATURE OF THE PROBLEM.

A REVIEW of the various techniques published during the past few years for the elucidation of chromosome structure shows that much attention is being paid to pre-treatment methods such as exposure to acid and ammonia fumes, maceration, digest with enzymes, the hot-water technique, and submitting the tissue in bulk to changes of temperature (Darlington, 1942; Kuwada, 1939; McKay and Clarke, 1946; Swanson, 1942). While the last method sometimes allows for a greater expansion of the spiraling chromonemata with increased visibility and inhibition of nucleic acid metabolism, pre-treatments as by ammonia fumes act in such a manner as to cause swelling and relaxing of the chromosome thread. The effects under certain circumstances often resemble those of low temperatures; these pretreatments must always remain suspect because of the obvious distortions that must accrue.

While much has been learned from these pre-treatment techniques a danger lies in the useful limit being passed and that more artifact than true fact is the result. This is particularly shown in a paper by Swanson (1942), giving details of heat treatment on *Tradescantia* where it was found that at low temperatures, 33.5° C., and high temperatures, 40–42° C., pronounced despiralization took place, but at the higher temperatures great irregularities obviously occurred for the author states that "other slides at these temperatures were so anomalous to be excluded from such consideration" (p. 463), and on p. 466 commenting on fig. 18, "*so far as can be determined*, and early diplotene configuration" (my italics), exemplify the need for care in the interpreting of these results: indeed, the figure mentioned presents a condition that in my experience appears to be in the process of dying; other figures seem in little better condition.

While we should not confine cytology to the level of a purely descriptive science, for to quote Thomas (1945), "the principles which underlie normal cell division and differentiation, however, are still far from being understood," yet there is a limit to which experimental methods can be applied for descriptive ends, chiasmata analysis, for instance, for while X-ray irradiation experiments may teach us much of the mechanism of chromosome division and change, translocation, and recombination, it is highly important in such studies that we should be able to trust our technique not to introduce changes and thus engender erroneous conclusions.

2. THE PROBLEMS INVOLVED IN MODERN METHODS.

It is difficult to arrive at a satisfactory conclusion about what is a really good preparation, and much has been learned by experiments undertaken with the object of ascertaining the shortcomings of the various media and processes used in micro-technique. A slide that was considered pleasing a quarter of a century ago is now known to be quite imperfect and even the criterion of comparing several different fixatives is liable to considerable doubt. Much also depends upon the subsequent processes, as pointed out by Maheshwari (1939) and Grigg (1938), in dealing with dioxan dehydration; this showed the fallacy of the generally accepted conclusion that a clear zone exists in the chromosome fibers around the nucleolus in the resting nucleus.

The great disadvantage of the almost universally used iron-aceto-carmine technique for plant smears is the great swelling produced by the use of uncontrolled acetic which causes the chromosomes to swell very greatly, in fact, often as much as 3-4 times greater than that seen in ordinary paraffin sections. Darlington (1931) states that the proportion in bulk between chromosomes fixed by medium Flemming and those fixed by McClintock's aceto-carmine is approximately as 4:1 and states that such difference exists in bulk fixations. I have not found quite such great disparity with sections prepared by the dioxan low melting-point paraffin continuous process (unpublished), for the usual paraffin with a melting-point at from 52 to 60° C. contracts chromosomes strongly. A paper by Shinke and Shigenaga (1933), on *Tradescantia reflexa* pollen mother-cells fixed with 30-35 p.c. acetic acid shows the chromosomes enormously swollen and blocking up the interchromosomal spaces, a condition, which on the basis of all the evidence available never exists in life.

The swelling of pollen mother-cell smears can be partly offset by a previous fixation in acetic alcohol but the use of a mixture with too high a concentration of acetic acid offsets to a great degree the advantages gained. This swelling may, sometimes, be useful in revealing internal architecture, but it is obvious that with structures so near the limits of visibility our caution with technique should be doubly alert. Thomas (1940), in an excellent criticism of the iron-aceto-carmine method, goes so far as to say, "Experience in this laboratory with the treatment of small chromosomes in various fruit species leads to the belief that what is needed is a *radical modification* of the fixation and mordanting methods used with aceto-carmine" (my italics), the suggestion also holds good for other types of material for in the past the carmine method has been regarded as the foundation-stone of smearing techniques used blindly without question. Other workers who have criticized aceto-carmine are La Cour (1941), who considers it not particularly selective for small chromosomes and that preparations often deteriorate when made permanent, and Zirkle (1940) who finds that many of the finer details of the chromosomes are destroyed by 45 p.c. acetic acid. White (1942) appears to consider pre-treatment techniques as stretching the chromosomes "somewhat roughly" and giving rise to artifactual pictures which he describes as a "dissection of a chromosome."

Techniques exist involving the use of other fixatives and stains, but like the former often require many hours to obtain complete staining density.

The method to be described has arisen from experiments undertaken by a process eliminating as far as possible the inherent shortcomings of the materials and processes used to produce a rapid efficient fixative/stain that will preserve the spatial arrangements of chromosome configurations, their proportion and external form, to effect optical differentiation of the internal structure of zygotene to diakinesis stages, and to further the study of chiasma formation.

Of the stains used two showed most promise, namely, hæmatoxylin and carmine, and early endeavours to use either as a counterstain to the other gave results that were encouraging. A few reports have appeared on the use of hæmatoxylin for smears and rapid staining (Beadle 1933 and Barrett 1932); the latter included too much acetic in his formula, and it is practically impossible to avoid a muddy cytoplasm with adequate staining of chromosomes with this technique; in addition very little internal structure is brought out. It would appear from Beadle's report that he was dissatisfied to a large extent with his method and seems to blame this for the inconsistency of his results. This concerned chiasma frequency at diakinesis and terminalization, and he states that the methods of both Darlington and himself swell the chromosomes considerably that the determination of whether chiasmata are actually terminal or not is more or less arbitrary. He appears to consider that there was a real difference in interpretation in this respect, but it would seem that if there were in reality a difference in interpretation a better technique would have been advantageous, on which point there appears with both authors to be some agreement. In the same paper Beadle comments also upon the importance of ascertaining whether diplotene "opening out" (chiasmata formation) is in the equational or reductional plane; in this connection he states that the technique used was unsatisfactory, and while it must be stressed that we cannot by any technique expect perfect results with a subject so near the limits of visibility of the optical microscope a closer consideration of this side of the question will bear fruit.

The swelling of chromatids by pre-treatment or high acetic acid fixation in certain instances reveals underlying structural units, but a useful limit to this process is very rapidly passed and the extremely bloated condition defeats its own end.

Another factor that is not always attained in a technique is sufficient transparency, as was pointed out for iron brazilin by Darlington (1933), who considers the stain too dense for the observation of essential side views. This is also true for the techniques of Beadle (1933) and Barret (1932). Kuwada (1939) in a review of chromosome structure figures *Tradescantia* root-tip nuclei prepared by a maceration method and stained with aceto-carmine (Aisma). This technique certainly shows structure not normally visible but the question arises as to what degree this structure may be *altered* apart from a general relaxing of what would seem, on the basis of modern knowledge, to be a linear accretion of protein chain molecules (Wrinch, 1934).

Johansen (1940) in a methyl-violet technique for which he claims "brevity, ease of manipulation, and certainty of obtaining sharply stained chromosomes from diaphase onwards," says the method is ordinarily useless for pre-diaphase stages. He appears to consider it may be useful for these early stages in

"certain plants," but expands the statement no further. The clue to this factor with violet-stained preparations is that gentian-, methyl-, and crystal-violets in their various methods of application are specific for the nucleic acid and leave the protein fibers relatively unaffected. Some plants do indeed appear to have a precocious disposition for nucleic acid metabolism at these early stages as compared with others but little internal structure is revealed in dia- to meta-phase by this method. The technique later to be described gives excellent preparations of early stages and good optical differentiation of internal structure at later conditions of growth due to the fact that like ordinary aceto-carmine it is largely specific for the protein thread, and only colours the attached nucleic acid later on in the process. It is no doubt the capacity of hæmatoxylin for staining the protein fibers that leads Johansen to praise Tuan's method (Johansen, 1940), where he describes the preparations to be of surpassing beauty.

The differential deposition of nucleic acid in different plants is well shown by a high-contrast gentian-violet technique (Grigg) for sections from dioxan dehydrated material, as yet unpublished, where the coating of the protein fibres is quite easy to follow.

3. THE REQUIREMENT OF AN EFFICIENT TECHNIQUE.

An ideal technique is obviously hard to find but careful qualitative experiments have yielded the results herewith presented. Transparency is attained especially in metaphase lateral figures which allows critical observations to be carried out through the entire cell (Figs. 19-24, pl. II). (These figures are printed densely to show detail in the diverging arms of the upper bivalent and for efficient reproduction, but similar structure is visible in the preparation in all chromosomes present, note also the lowest-but-one chromosome in fig. 23).

The requirements of an efficient smear technique appear to be as follows :

(a) Reasonably rapid, viz. a 25-35-minute process combined with good and efficient fixation within that time suitable for permanent mounting in Balsam for high-power observations.

(b) A technique that allows the retention of the greater proportion of the smeared cells on the slide *without* operations of extreme delicacy that preclude efficient washing in the changing solutions.

(c) Transparency in finished preparation for efficient observation of lateral views (*vide* remarks of Darlington comparing iron brazilin with gentian violet).

(d) Internal optical differentiation of chromonematic thread especially during diplotene and diakinesis (*vide* remarks of Beadle), not clearly attainable with gentian violet and often indifferent with aceto-carmine.

(e) A stain that will have a reasonable sharpness throughout any structure it colours *if* that structure possesses a sharp boundary, and will have no tendency to a diminution in density towards the periphery of any body so treated assuming that it possesses a similar constitution throughout, in contrast to what is often found with ordinary iron-alum-hæmatoxylin after Heidenhain and with gentian violet caused by the process of destaining.

(f) A technique that will allow no changes to take place in the preparation after immersion in fluid balsam in the operation of sealing down which is much more common than is generally suspected.

4. CONSIDERATION OF RESULTS.

Plates I and II show the result of this new technique. Anthers were cut in half, one portion being smeared and the other being passed through the paraffin process for purposes of comparison. It was found with *Lilium* material that the anthers are not always at the same stage of growth, and the six may range through 2-3 hours or more of development in one inflorescence. This renders the partition of them into two necessary for correct consideration of two techniques. This is a common occurrence with rapidly developing plants although with species possessing long anthers as the Liliaceæ the P.M.Cs. situated near the ends of the long cylindrical loci are usually later in development than those towards the centre. However, it is particularly noticeable in cool weather that all stages from pachetene to metaphase-2 may appear mixed up in one locus, and this is very useful for ascertaining exactly the relative effects of a technique on one slide. Under these conditions the chromosomes sometimes appear like preparations subjected to moderate cold pre-treatment, the heterochromatin being more visible owing to the presence of less nucleic acid. The chromosomes in cool grown material are often seen to have a looser structure with larger outlines than those at corresponding stages in rapidly growing material. It would appear that this is due to a more leisurely growth and not to the effect of the special technique for similar changes are observable in the same material prepared by the paraffin process.

An examination of the photomicrographs will show the absence of any serious swelling or contraction, and internal optical differentiation is clearly visible in the smeared cells. The boundaries of the chromonemata are clearly defined and sharp with no appearance of a peripheral diminution in staining density. There appears to be about a 5 p.c. contraction in the cytoplasm away from the cell wall due to dehydration effects; this may affect the size of the chromosome plate but it is not considered at all serious.

In the fixation of the various stages it was found that the chromosomes showed a somewhat fuller outline than those from identical material passed through the paraffin process. This is attributable to the absence of heat treatment. However, at full diakinesis the vacuolation as described by Darlington (1938) was prone to appear, but *only* at this stage. Indeed, with many fixatives this condition is hard to avoid and is probably due, partly at least, to the structural condition of the bivalents.

The chromosome thread is composed of chains of molecules (Wrinch, 1934), and at diakinesis these are at their greatest expansion, when, no doubt, they are in a comparatively quiescent condition or at least undergoing a slight pause at the end of one cycle of changes and the beginning of the next. At this stage they would be under little strain as contrasted with say pachetene or metaphase, and under these conditions the molecular framework would be unable to resist

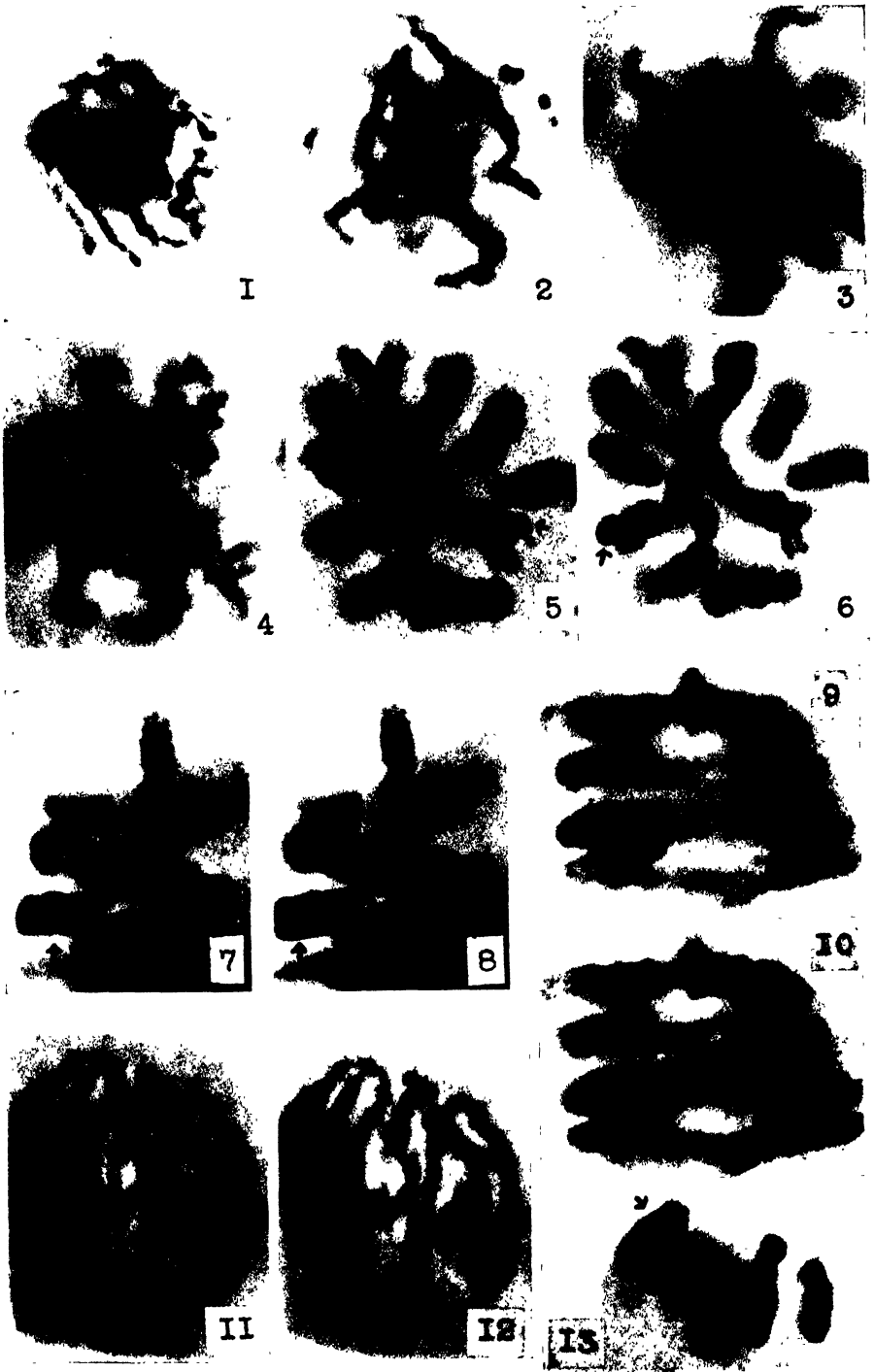
the vacuolation effect of the fixative as also holds with cytoplasm (Grigg, unpublished work), and the observed effect would be expected. This technique does not produce vacuolation effects in metaphase chromosomes as Darlington's (1938) text-figs. 1 and 2, p. 617, show. Different fixations are required for the changing stages of chromosome division, but we might ask ourselves whether we could expect to fix the diakinetid bivalent in a natural structural form, a similar problem as with cytoplasm, for, although we can show degrees of change that must exist, the most we can obtain is only an *intensification* of a natural condition.

Figs. 5 and 6 show an example of a cell at diakinesis exhibiting this effect in certain parts of the bivalents and not in others (arrows indicate circular cavities). This is really a shrinkage effect caused by the extraction of natural water; it is often more noticeable in the extended arms of the bivalents than in the middle portions where the protein threads (chromonemata) are likely to be under a small degree of restrictive tension (inter-chiasmata repulsion). These very small vacuoles can often be traced within the bivalents especially between the chromatids, but have little displacement value in these parts of the chromosomes.

Kuwada (1939), p. 226, finds the internal structure of the chromosomes at diplotene is obscured and considers this obscurity may have some significance, and goes on to say, "In plants, the double nature of the elements is lost to view before reaching the diakinesis"; with the technique herein presented this internal structure is usually visible throughout the cycle. This author also depicts (fig. 8) *Tradescantia* P.M.Cs., presumably pre-treated, which show the chromosome thread as a very much thinner structure than those prepared by me: a certain amount of distortion is evident as the preparation was probably crushed and pre-treated as are most of those illustrated in the paper. Pronounced spiralization is visible including "minor spirals." Chromosome threads are prone to spiralization, indeed it is considered to be a necessary prerequisite of their existence in higher organisms and we must consider the possibility of a technique *intensifying* this effect or even of inaugurating a spiralizing *before* it had begun in life owing to a pre-mature contraction of the molecular chain structure due to the technique. In short, spiralizing is a varying effect of the molecular structure of the chromosome following periodic phases from the molecular spiral of Darlington to the relic spiral of the same author.

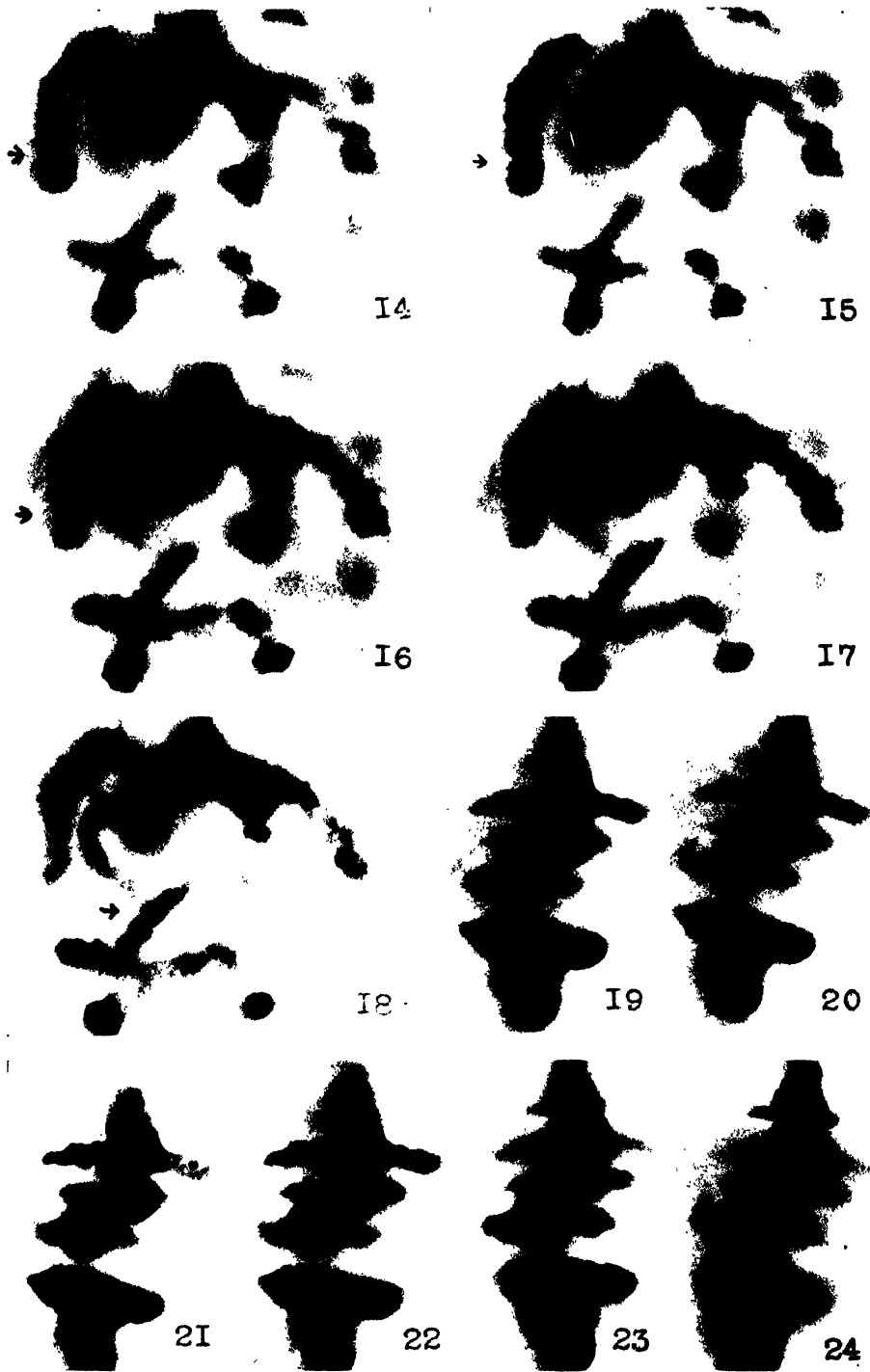
The use of ordinary acetic-alcohol produced great vacuolation effects in both diakinetid and metaphase chromosomes, but if the alcohol and acetic content are decreased as herein indicated this vacuolation is confined to full diakinesis. This effect is found with formalin-alcohol and formalin-acetic-alcohol if the concentration of alcohol is too high.

On the face of all the evidence available it would appear that pre-treatments by acid and other vapours precipitates and to a certain extent fixes the chromosome thread and prevents the vacuolation effect of the fluid fixation. The "relaxing" effect of pre-treatments such as hot water have a slightly similar action but also tend to straighten out the chromosome thread by upsetting the torsion inherent in the structure. Hot water tends also to dissociate or dissolve the matrix which would also permit this unravelling. Enzymes act in a different



Figs. 1—13.

[To face p. 30].



Figs. 11-24.

way (McKay and Clarke, 1946), by digestion of the softer parts of the chromosomes leaving the basic thread till last.

The diakinetik chromosome has all the appearance of an isolated system in a condition of greatest equilibrium both spatially and internally. Strains and repulsions are balanced out. The water content is obviously high for under conditions of inefficient fixation the vacuoles occupy a greater proportion of their bulk, and may even amount to 80–90 p.c. of their entire volume. Darlington and La Cour (1938) state that this effect, described by them as “bubbles,” can be produced in its clearest form by fixation with acetic-alcohol and also by the use of Medium Flemming, with the latter I have found this bubbling due to the use of alcohol for dehydration, but when the material is passed through a slow dioxan process this does not occur.

Chromosomes at this stage appear to possess considerable rigidity, for in spite of vacuolation the external form is little changed: we must not, however, in this connection, forget D'Arcy Thompson (1942), who says, “the grosser analogies may lead us astray.” He considers that apart from electro-static effects surface tension is a prime cause of the great rigidity of minute fluid and semi-fluid systems. Also, as the diakinetik bivalent possesses a framework of comparatively strong protein threads this preservation of external form is to be understood.

As indicated above this tendency to become vacuolate was greatest at full diakinesis, but just as soon as the centromeres began their progression this tendency disappeared in the parts adjacent to these bodies and the extended arms immediately assumed their proper appearance of spirality (figs. 19–24). The vacuolation, however, persisted in the distal unstrained portions of the bivalents. At full metaphase and anaphase it was never evident in these preparations and any technique that produces vacuolation at these stages as illustrated by Darlington (1938), must be considered defective.

The chromonemata can be clearly seen to be much slenderer than in preparations pre-treated with acid vapours, so the possibility that these cells present a more natural form is apparent. In perfectly prepared diakinetik bivalents even in the static portion on the equatorial plate no vacuolation whatever is visible (figs. 7 and 8, upper bivalent). At the arrow in the lower left-hand chromosome the crossing-over of chromonemata is clearly visible.

In view of the aforementioned facts we can regard meiotic bivalents at diakinesis somewhat in the manner we view cytoplasm, as a very fluid structure often showing, except for boundaries, the distorting effects of the technique.

5. SCHEDULE FOR IMPROVED SMEAR TECHNIQUE.

The following formula was first perfected thirty months ago, and as far as can be judged these original preparations exhibit no observable change; the carmine content of the combination is still contributing its specificity to the stain; it would appear to be reasonably permanent. Several batches of ordinary aceto-carmine preparations rendered permanent by various methods for controls at the time show so much serious deterioration as to render them useless.

Procedure :

- (1) Smear in the usual way with fresh material and allow to desiccate for a few seconds (blowing assists this process).
- (2) Invert slide over following pre-fixative :

80 p.c. alcohol	...	...	...	...	80 parts.
Glacial acetic acid	...	...	...	...	20 parts.

 Allow to act for 20–30 minutes according to material.
- (3) Drain and flood with stain/fixative *used fresh* :

Solution a.

0.5 p.c. hæmatoxylin (about 2–3 weeks old)	1 part.
4.0 p.c. iron alum (fresh)	0.751 to 1 part (or less).
Absolute alcohol	2 parts.
Glacial acetic acid	0.5 to 0.75.

Add to above equal quantity of :

Solution b.

12.5–20 p.c. aqueous solution acetic plus carmine to saturation boiled and filtered. (*Concentration of acetic should be approximately near to solution a.*)

- (4) Wash off 50 p.c. alcohol ; 80 p.c. ; 100 p.c. : care should be taken with these steps to ensure the acetic being completely cleared from slide.
- (5) At least three changes of alcohol/xylol should be used in ascending concentrations of xylol ; but it is undesirable to pass into pure xylol but mount from about 60 p.c. xylol plus 40 p.c. alcohol.

Solution *b* can be added in a lower proportion than 50 p.c. to the stain/fixative (3), in which case the concentration of alcohol goes up. Stage 2 should be so varied that stages 2 and 3 together are completed in about 25–35 minutes ; some material appears to require more pre-fixation than others and less staining, but the above schedule has been used to produce the slides from which the photomicrographs were taken and can profitably be taken as a starting point for most work. The stain should be used within 5–6 hours of making up as a precipitate occurs and staining capacity is lost on filtration.

In general the acetic concentration of the stain should be lower than that of the initial fixative as likewise that of the alcohol content. This conforms to my contention that a fixative should represent a steadily diminishing factor. With regard to the initial pre-fixation in acetic-alcohol this must be varied to suit different material but it is necessary to keep the concentration of acetic acid and alcohol somewhat lower than with the usual mixtures. Baker (1938) found a diminution of alcohol content advantageous in studying spermatogenesis of *Salamander*, for he states, "Towards the centre of the piece of tissue this effect is less marked (on cytoplasm) because the alcohol has been diluted by the water contained in the tissue."

Owing to the delicacy of the results with this technique each batch of new material should be regarded as an experiment, and consideration towards this end should be borne in mind. This becomes particularly true as the minimum time of fixation is used thus mitigating the evils of contraction of chromosome configurations so noticeable with over-fixation. It is also noticeable that P.M.Cs. surrounded with anther slime do not take up the stain so readily as isolated cells, but this, at first seemingly a disadvantage, supplies a valuable series of cells stained in all gradations; also the best results are obtained if staining is somewhat lighter than is usual with material for observation with white light, the necessary contrast being obtained with dark-green filter combinations, by which means the maximum advantage is obtained with this technique.

It is not possible to obtain efficient fixation in a lesser time than that indicated with acetic alcohol, but it may be possible with other fluids. However, "only 20-30 seconds" in a variation of Flemming after Geitler as recommended by Levan (1937), is far too brief. His illustration (fig. 14, p. 349) shows a series of metaphase bivalents exhibiting a condition of insufficient fixation; the arms of the bivalents between the centromeres and joined portions of the chromosomes show a general distortion and shrinkage. Contraction and distortion can occur, as I have ascertained, *after* mounting while the balsam is still fluid under conditions of insufficient fixation.

The process taking place with the application of this stain seems obscure. Possibly the alcohol acts as a mordant; staining is usually very poor with a very much lower alcohol content than that indicated, and it certainly prevents the swelling of chromosomes as has been proved by careful measurements carried out as soon as they become visible. It is possible to use the hæmatoxylin solution alone suitably diluted as would be the case if the carmine solution were added, but a foggy cytoplasm results and internal differentiation of the chromosomes is lost. The carmine then appears to inhibit the cytoplasm from taking up the hæmatoxylin and, adding its own specificity to the solution, produces the results obtained.

On balance this technique has not proved easy to handle but, when good preparations are obtained, inner detail of chromosomes is revealed in a manner unobtainable by any other method.

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DESCRIPTION OF PLATES.

ALL POLLEN MOTHER-CELLS.

PLATE I.

- Fig. 1.—*Lilium candidum*. Pachetene. Spiral coiling just beginning to show. $\times 1450$. Wratten filters 74 plus 65a.
- Fig. 2.—*Lilium candidum*. Pachetene/Diplotene. Note opening out of chromonemata at 12 o'clock. In the preparation it is possible to follow these threads nearly the entire length of this chromosome. $\times 1450$. Wratten filters 74 plus 65a.
- Fig. 3.—*Lilium Henryii*. Full diplotene. Loosening out of threads taking place. Chromosomes still contracted around nucleolus. A second nucleolus is visible to the right. $\times 1450$. Wratten filters 62 plus 65a.
- Fig. 4.—*Lilium Henryii*. Slightly later stage than fig. 3. Chromosomes contracting in length and width of spirals increasing. A cross-over is visible opposite arrow. Note wide spiral in bivalent at 6 o'clock. $\times 1450$. Wratten filters 74 plus 65a.
- Figs. 5 and 6.—*Lilium candidum*. Full diakinesis. Two foci of same cell. Note spirality of single threads in many places and cross-over in bivalent at 6 o'clock in fig. 5. $\times 1500$. 4, Wratten filters 74 plus 65a; 5, Wratten filters 74 plus 45a.
- Figs. 7 and 8.—*Lilium candidum*. Full diakinesis. Two foci of same cell. A little later than 5 and 6. Bivalent at 12 o'clock shows subterminal chiasmata with upper chromatid in focus only, internal coiling of chromonemata clearly visible. Fig. 8 is at a focus of 0.25μ lower than 7. At the base of this arm in fig. 8 the chromonemata can be seen to be strained out of their circular position of equilibrium by proximity to the chiasma. In lower left-hand bivalent above arrows in both figs. crossing-over is seen. $\times 1750$. Wratten filters 74 plus 65a.
- Figs. 9 and 10.—*Lilium Henryii*. Metaphase-I. Two foci of same cell. The double coiled nature of each chromosome arm is clearly visible. $\times 1750$. Wratten filters 62 plus 65a.
- Figs. 11 and 12.—*Lilium candidum*. Telophase-2. Two foci of same cell 0.25μ apart. Spirals strongly shown. Evidence of the "molecular" spiral visible in centre of fig. 12. $\times 1750$. Wratten filters 61 plus 45a.
- Fig. 13.—*Lilium Henryii*. Anaphase-1. Double-coiled structure visible at arrow. $\times 1750$. Wratten filters 62 plus 65a.

PLATE II.

- Figs. 14–18.—*Lilium candidum*. Late diplotene. A cross-over is seen at arrow in 14–16. Structure should be studied at arrow in fig. 18. This particular phase of diplotene is extremely brief, and follows on the condition shown in fig. 4, pl. I. $\times 1750$. Wratten filters 74 plus 65a.
- Figs. 19–24.—*Lilium candidum*. Pro/Metaphase-1. Serial optical sections through same cell. The structure of the diverging arms of the bivalents should be noted. These were printed darkly to show this structure but much also is visible in the negatives and preparation in the other portions of the chromosomes, particularly the lowest-but-one in figs. 22–23. The staining capacity of the diverging arms is always much less than the unstretched central portions which is, no doubt, due to their more condensed condition. The centromeres are just visible. $\times 1750$. Wratten filters 74 plus 65a.

V.—THE ORGANIZATION OF THE MALIGNANT CELL.

PART I.—PROTOPLASMIC DISORGANIZATION IN TUMOUR CELLS.

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1. INTRODUCTION.

IN this paper an attempt is made to relate some of the salient features of certain neoplasms to the submicroscopic disorganization of protoplasm. Unlike normal organismic components which are effectively integrated into an operative entity, tumours are not under the complete control of the organisms in which they occur. Neoplasia result from failures in the internal co-ordination of organisms.

Disorganization may become apparent at any of the levels of biological order and may proceed so far as to result in the deaths of the intrinsically affected cells. Short of this culmination, malignant cells are those least organized and provide the greatest contrast between organized and disorganized tissues. Since failure of organization is a potential hazard in all organized systems, the occurrence of neoplasia in diverse groups of animals is to be expected. Tumours have been observed in all the main classes of vertebrates and in some kinds of invertebrates, including molluscs (Smith, 1934), insects and annelids (Needham, 1942). Atypical and continued proliferation of mesenchyme cells which kills affected sea-urchin larvæ has been experimentally produced by Tschakhotines (1938). Abnormal protozoa have been produced by treatment with carcinogenic agents and Mottram (1942) has drawn a close parallel between cancerous cells and treated *Paramecia*. White (1944) has described dedifferentiated, rapidly growing and transplantable neoplasia in plants.

The biological characteristics of comparable neoplasia in different groups of animals appear to be similar even though the detailed chemical and physical mechanisms concerned may vary. Neoplastic cells are essentially similar to normal ones in most of their biological processes and it appears that malignancy is the result of some aberration of a fundamental cellular process or substratum which leads to disorganized growth.

2. BIOLOGICAL ORGANIZATION AND DISORGANIZATION.

The normal organization of an animal depends on the harmonious integration of its parts during development and on the continued mutual regulation of the component parts of the adult. The different grades of biological order fall

naturally into a series of levels of increasing organization each of which is dependent upon inferior grades for the conditions necessary for its own development. Since growth, histogenesis, organogenesis and the co-ordination of organs are mutually independent processes, and as cellular growth and differentiation can be dissociated (Needham, 1942), effective organization at a particular level does not necessarily lead to proper organization at the succeeding level. The characteristics of aberrations produced by disturbances of organization are determined by the ontogenetic stage at which they are initiated and by the particular level of organization that is primarily affected. Such considerations provide a partial basis for a natural classification of neoplasia. Disorganization may result from inco-ordinations in various classes of organismic components including organs, complex and simple tissues, and similar cells.

Terata provide examples of the effects of inadequate organization during early development. As terata have been produced by the implantation of organizers into normal embryos, it has been suggested (Needham, 1942) that the natural occurrence of these abnormalities can be ascribed to the presence of supernumerary organizers. Failure of organization in these instances takes place at the level of co-ordination of organs. The organs are usually normal except at zones of fusion between incipient individuals but they are not co-ordinated to form a single normal individual.

Teratomata differ from terata in that they are contained in the bodies of otherwise normal organisms whereas terata, *sensu stricto*, are organisms of abnormal, incomplete or replicated morphology. Teratomata are characterized by the absence of controlled regional differentiation, unlike terata, and are complex conglomerations of histologically differentiated tissues which may form incompletely developed organs. They illustrate faulty co-ordination of tissues.

Teratomata develop most frequently from gonad tissues, presumably from germ cells, but embryonic cell "rests" are not uncommon in mammals (Ewing, 1928). Pluripotent germ and embryonic cells could give rise concurrently to many kinds of histologically different cells such as are found in some tumours. The cell "rest" theory, although it does not account for the initiation of the neoplastic transformation, may therefore partly explain the polymorphic histology of teratomata and mixed cell tumours. The latter might also arise through metaplastic transformations of cells of certain types.

In complex tissues the elements of the various cell types are arranged in particular patterns. Similarly, in simple tissues, with a single dominant cell type, like cells are orderly arranged in regard to each other and to the stroma and vessels. Disorganization of the normal relations between the cells of a tissue results in structural aberrations even though, in benign tumours, the individual cells may appear normal by microscopic observation. Simple tumours are due to the transformation of one kind of cell. Benign tumours differ from malignant ones in their retention of a greater semblance of normal structure, whereas malignant cells are usually irregularly arranged and less differentiated. However, both these kinds of growths illustrate subnormal co-ordination of cells at the level of tissue formation.

Although the demarcations between the various types of tumours are necessarily arbitrary because of the frequency of growths which are intermediate

in structure and behaviour, the distinctions between them are none the less real. The organization of the several levels of biological order must at some point be dependent upon the properties of cells. The varying grades of disorganization shown by "true" neoplasia, that is, ones consisting of anaplastic cells, appear to be related to corresponding degrees of internal disorganization in their component cells. Malignant cells constitute a convenient focal point for an analysis of the problems of tumour growth since of all cells they are the most changed from a normal co-operative mode of life and present the sharpest antithesis between biological organization and disorganization. Other kinds of anaplastic tumours appear to differ quantitatively rather than qualitatively from malignant tumours and it should, therefore, be possible to relate to them conclusions reached in a study of the latter. Accordingly, greater consideration will be given to malignant rather than to benign tumours.

3. THE INTRACELLULAR LOCALIZATION OF MALIGNANCY.

The disorganization of an organism might theoretically result from either the absence of the factors responsible for normal organization or an inability of modified cells to respond to these factors. Although a few instances are known in which abnormal embryonic growths are produced by failures of the organizing agencies, there is little doubt that other neoplasia arise through modifications in the neoplastic cells themselves. Some of the evidence for this conclusion is as follows. The facts that only one or at most a small number of cells are initially cancerous and that the great majority of the cells of a tumour-bearing animal are normal point to the presence of the regulatory factors. In addition, serial implantations of tumours are commonly successful when made into homologous animals previously without tumours. It appears that the agencies responsible for the general organization of an affected animal are still functional and that malignant cells have lost the power of response to these factors.

Malignant changes have also been induced in cells grown *in vitro* by carcinogenic hydrocarbons (Earle, 1943), as shown by the development of tumours at the sites of implantation of treated cells into homologous animals (Earle and Nettleship, 1943) and the histological characteristics of these tumours (Nettleship and Earle, 1943). These researches show that in general it is unnecessary to postulate a systemic cause of malignancy other than the possible production of carcinogenic agents.

Although the agent initiating carcinogenesis may in some instances be extrinsic to an affected cell, the specific response of this cell must be determined by innate factors. The proximate cause of malignant growth resides in affected cells themselves since the malignant state is inherited by successive generations of cells growing *in vivo* or *in vitro*. The inability of an organism to control malignant growth should, therefore, be attributed to factors intrinsic to the affected cells. The central problem of malignancy appears to be that of the nature of the transformation undergone by the modified cell. As malignant cells are characterized by a complex pattern of associated properties it is important to know which of these is fundamental since such a property might provide a key to the etiology and nature of the malignant state.

4. THE INTER-RELATIONSHIPS OF THE CHIEF BIOLOGICAL CHARACTERISTICS OF MALIGNANT CELLS.

Neoplasia are commonly distinguished and graded by their biological and morphological characteristics as their importance in pathology is due to their biological behaviour which is in turn related to their histological appearance. Malignant tissue is less integrated into the morphological and physiological organization of an affected organism than are other neoplastic tissues and is "anaplastic" according to the original usage of this term (Ewing, 1928).

A tumour that is not highly malignant by histological criteria may nevertheless rapidly kill its "host" and is, therefore, clinically malignant. Adenoma of the thyroid gland falls into this category. In this kind of tumour the close proximity of the neoplastic cells to abundant thin-walled vessels provides unusually favourable circumstances for their passage into the latter. Not infrequent metastases therefore occur. In the absence of such peculiar local conditions tumours of comparable histological anaplasia are not so clinically malignant. Apart from such instances of topographical malignancy, the assessment of malignancy by the degree of morphological dedifferentiation is generally accepted as a valid procedure. Topographic malignancy will not be considered in the following discussion since it is primarily determined by local environmental factors.

The chief features of malignant cells include continual proliferation, autonomous growth, invasion and infiltration, metastases, and dedifferentiation. These properties are causally interconnected and underlie the operative pathological effects of malignant tumours which include, for example, interference with the structure and functions and the destruction of normal tissues and organs, and the systemic absorption of toxic products. Such subsidiary pathological effects will, therefore, not be considered further in this paper.

In general, malignancy is usually agreed to be correlated with the degree of histological anaplasia shown by a given tumour. The most malignant cells are precisely those that are least differentiated (Ewing, 1928; Lockhart-Mummery, 1984). Cell divisions are most frequent in the least differentiated of normal tissues and, conversely, the most highly differentiated tissues show the fewest cell divisions. Furthermore, differentiated cells become less differentiated preparatory to division but the daughter cells return to the differentiated state. A correlation exists between the incidence of mitosis and the degree of differentiation shown by a specified tissue whether it is normal or malignant (Berrill, 1935, 1943; Needham, 1942; Reimann, 1938). Tar-induced malignant tumours grow faster than homologous benign tumours in mice (Mottram, 1984). It has been suggested that malignant cells reproduce abnormally quickly. However, they grow no faster than foetal tissues or regenerating liver (Ewing, 1928) or than normal cells *in vitro* (Berrill, 1943).

With an increased rate of proliferation the phase of pre-division (cytokinetic) dedifferentiation becomes more pronounced in a tissue as a whole. If the interkinetic periods are too short for full redifferentiation, cytokinetic dedifferentiation may contribute to an appreciable extent to the manifested state of dedifferentiation. The histological appearance of a given tumour will

depend upon both the primary anaplastic and the cytokinetic dedifferentiation. The former also influences the latter through its correlation with cell division. In comparing tumours it is, therefore, necessary to evaluate the relative importance of these factors.

The morphogenetic control of its component parts by an organism is achieved through orderly cell formation, histogenesis, organogenesis and co-ordination of organs. The organization of an animal depends upon the specialization of its component parts, especially on that of the cells themselves which are the basic units of organization. The morphological and chemical differentiation of a cell underlie and make possible its specialized role. Then, insofar as cells lose their capacity for differentiation, they cannot be integrated into the economy of an organism. Despecialized, malignant cells which remain dedifferentiated and continue to proliferate therefore manifest unorganized and autonomous growth.

The formation of metastases is ancillary to the properties of invasion and infiltration since secondary growths arise from cells which become detached from the parent tumour. The spread of malignant cells into normal tissues and tissue spaces is partly due to rapid proliferation and consequent peripheral pressure of growth and to the autonomous behaviour of the tumour cells. The morphological plasticity of certain malignant cells (Ewing, 1928) which is related to the loss of formed structure consequent upon dedifferentiation also accounts in part for the properties of invasion and infiltration. Mottram (1942) has described the abnormally flexible *Paramecia* produced by treatment with benzpyrene.

The dedifferentiation of malignant cells would seem to be their most fundamental property at the cellular level since their other chief characteristics listed above can be derived from this state. Several workers have concluded that the fundamental quality of malignant cells is that of failure to differentiate normally (Reimann, 1938). The sequelæ of rapid continual cell division and autonomous growth are the direct outcome of dedifferentiation. The invasive and infiltrative abilities of malignant cells are determined by the preceding qualities and that of morphological plasticity.

Cells of a particular kind in a given species of animal tend to have a characteristic size range which varies within relatively narrow limits. This generalization applies to dividing normal or malignant cells. Cell division and growth must, therefore, be inter-related since cells of the same kind would otherwise vary greatly in size. The existence of a standard size range for a given kind of cell implies that division results if a cell exceeds a critical size and that it does not otherwise take place. The dependence of cell division upon the amount of protoplasm available has been experimentally demonstrated (Phelps, 1926; Looper, 1928).

Cell division usually involves the synthesis of protoplasm except when it occurs during the segmentation of an egg cell. The hypothesis that malignancy is due to an irreversible drive to cell division is untenable in its simple form, that is, the primacy of division, since division is conditional upon adequate growth. On the other hand, a continual synthesis of protoplasm in excess of replacement needs would result in repeated divisions. Malignancy might, therefore, be due

to a ceaseless synthesis of protoplasm resulting from the unregulated reproduction of one or more of its essential components and the co-ordinated reproduction of other components in an attempt to attain equilibrium (Berrill, 1949). The antagonism between cell differentiation and mitosis may be due to the imposition of a definite structural pattern upon the cell as well as to the diversion of cell processes in a specialized direction.

5. CELLULAR SPECIALIZATION AND ANAPLASIA.

The discovery of viruses has modified the conception of the cell as the ultimate unit of living matter but nevertheless the cell can still be regarded as the basic unit of metazoan organization. Every variety of cell has its own special role and fits into the integrated activities and structure of the whole organism. Cellular "specialization" connotes the adaptation of a cell to the performance of a specific role in the economy of an organism. The counterpart of specialization, that is, the correlated changes within the cell itself, is indicated by cellular "differentiation." Specialization is dependent upon the appropriate differentiation of the cell concerned.

The performance of a specialized role involves modifications in the properties and nature of a particular cell, in comparison with a generalized unspecialized cell, which vary according to the nature of the specialization. Since, however, form and function are inseparably correlated, especially at the colloidal and molecular levels, the morphological and biochemical differentiation of a cell should be regarded as inter-related aspects of the particular state of its protoplasm.

Structural differentiation is the morphological aspect of cellular specialization and therefore affords an index of the nature and degree of the specialization. Chemo-differentiation bears a parallel relation to cell specialization. The assessment of the degree of morphological differentiation of a cell is based upon the state of the visible structural features associated with its characteristic specialization. Different kinds of cell which are fully and therefore comparably specialized vary greatly in their respective states of differentiation, that is, in observable structural complexity.

The failure of any particular kind of cell to fulfil its normal role results in a breakdown of the co-ordination of an organism. Hansemann's original conception of anaplasia included physiological as well as morphological changes; he emphasized that morphological signs of anaplasia indicate the extent to which a modified cell is not regulated by the organism. Anaplastic cells do not adequately perform the specialized roles of the cells from which they are derived so that anaplasia can be regarded as a degeneration from a specialized condition. It is not, however, accompanied by a restoration of pluripotency if the cell originally affected were fully specialized. Anaplastic cells are not equivalent to unspecialized cells but are despecialized cells with (usually) no further capacity for full development or specialization. However, unspecialized cells may become anaplastic, such as those giving rise to teratomata, and produce several histological kinds of cells concurrently. These derived cells are themselves anaplastic and do not become fully specialized. The final result is the

same whether the parent cells are specialized or unspecialized because the derived cells are incapable of full specialization. Despecialization and dedifferentiation are probably best regarded as subnormal specialization and differentiation respectively. Since specialization depends on adequate differentiation, anaplasia results from inadequate differentiation. An approach to the more fundamental causes of malignancy may, therefore, be made through a study of cellular differentiation. Anaplasia is usually assessed by the degree of the loss of visible morphological differentiation. It is, therefore, more noticeable in highly morphologically differentiated cells than in less well differentiated though equally specialized ones.

6. CELLULAR DIFFERENTIATION AND DEDIFFERENTIATION.

Cellular differentiation can be broadly defined as the manifestation of distinctive qualities in a cell. All differentiations are the results of changes in living protoplasm even when diagnostic histological changes are directly due to changes in metaplasia.

The nuclei of the different kinds of cells in an organism usually show little morphological variation. The modified morphology of the nuclei of certain differentiated cells, for example, leucocytes, is not directly associated with a qualitatively more heterogeneous internal structure. The nuclear differentiations of cells with polyploid nuclei, diplotene or polytene chromosomes, consist essentially of the replication of similar parts. These differentiations occur in both normal and neoplastic cells (Bieseke *et al.*, 1942) and do not affect the specific histological type of the cells.

During development nuclear differentiations are almost invariably trivial whereas the increased complexity of fully differentiated cells consists of changes in the cytoplasm. The occurrence of differentiation without cell formation has been recorded by several workers (Lillie, 1902 ; Scott, 1906) so that these two processes are mutually independent. On the whole, cellular differentiation is due to changes in cytoplasmic components and constituents and the role of the nucleus is subsidiary (Child, 1940 ; Harrison, 1940 ; Jennings, 1940).

Cellular differentiations are either of metaplastic nature and lacking viability or are transformations of living protoplasm. Temporary differentiations are more easily recognized to be modifications of living protoplasm than are more permanent ones, for example, asters and cilia respectively. Metaplastic differentiations such as fat droplets are obviously of lesser importance in relation to cellular organization than differentiations directly involving living protoplasm.

Dedifferentiation like differentiation is essentially a cytoplasmic phenomenon. The anomalous dedifferentiation of malignant cells might have its inception in either the nucleus or cytoplasm but, as it reflects the degree of organization of the latter, any causative factors of nuclear origin resulting in anaplasia manifest themselves through the cytoplasm. Cytoplasmic factors are, therefore, to be considered the operative or proximate, but not necessarily the initial or primary, intracellular causes of malignancy. The occurrence of hereditary tumours

indicates that the cytoplasmic changes are sometimes at least determined by nuclear factors.

The cytoplasmic site of dedifferentiation suggests that the malignant transformation can be ascribed to a failure of some system in living spongioplasm. The question arises as to whether or not this change is invariably preceded by a nuclear factor. To decide this point it is necessary to know to what extent cellular differentiation is controlled by the nucleus. There are two aspects to this problem, namely, that of the initiation of differentiation and that of its inheritance by somatic cells. Malignancy appears to be due to a persistent failure of normal differentiation and its pathological importance lies in its inheritance by proliferating cells.

The transmission of hereditary characters between organisms may be compared with the inheritance of differentiation by a lineage of specialized somatic cells in an individual organism. The chief site of modifications and control in the former type of inheritance is undoubtedly intranuclear. On the other hand, since all the somatic cells of an organism appear to have the same karyogenetic equipment, apart from sporadic mutations, somatic inheritance cannot be explained by controlled variations in the genotype. There appear to be two possible derivations of cytoplasmic differentiation, namely, that it is either a purely cytoplasmic phenomenon or is under the indirect control of the nucleus and results from interactions between this organelle and the cytoplasm. There is little doubt that the second alternative is correct.

7. THE AUTONOMY OF CYTOPLASM.

The subject of cytoplasmic inheritance has been reviewed recently by several workers including Darlington (1944) and Wright (1941, 1945), who agree that the authenticity of this phenomenon is well established. Darlington (1944) has enumerated three levels of inheritance, the nuclear system (karyogenes), the cytoplasmic systems of plastids (plastogenes) and the "undefined residue of heredity" (including plasmagenes). The evidence for cytoplasmic inheritance is more thoroughly established for plants than animals, largely because of the presence of plastogenes in the former. Most of this evidence has been gained by investigations of the differences between reciprocal crosses.

Plastogenetic inheritance has been studied in, for example, *Oenothera* and several cereals. Plastids are usually under the control of the nucleus but in maize and some other plants mutated plastids are autonomous. Instances of cytoplasmic inheritance are known in protozoa (De Garis, 1935; Kimball, 1939; Sonneborn, 1943; Sonneborn and Lynch, 1934), echinoderms (Moore, 1933), insects (Goldschmidt, 1938), amphibia (Hadorn, 1937; Hamburger, 1936; Moore, 1941), and mammals (Fortuyn, 1931; Law, 1938). In view of the induction of some tumours by viruses it is interesting that the distinctions between viruses and plasmagenes are tenuous in certain border-line instances, for example, in *Datura* and potatoes (Salaman and Le Pelley, 1930; Darlington, 1944).

Although heredity mechanisms are primarily controlled by the nucleus, cytoplasm has a certain degree of autonomy in this respect and, in particular,

has the faculty of reproducing itself relatively true to type. The fundamental role of proteins in the constitution of protoplasm is commonly accepted to-day. Because of their complex structure it appears that the formation of specific proteins can be achieved only through autosynthesis at some stage in which an existing molecule of protein acts as a model.

The chromosomes control highly specific characters, for example, in *Neurospora* (Beadle and Tatum, 1945), and form specific substances. Investigations on nuclear inductors have established that they can pass to the cytoplasm through an intact nuclear membrane (Hämmerling, 1935 ; Stern, 1937, 1938). Although proteins of nuclear origin could reach the cytoplasm during nuclear division it is unlikely that the immediate karyogene products are proteins since certain fully differentiated post-mitotic cells continue to live indefinitely. On the other hand, nuclear inductors are almost certainly not genes as such because, if they were, nuclear hereditary factors would be obscured by cytoplasmic ones unless the inductors were rapidly destroyed (Wright, 1941).

The specificity of proteins as determined by serological methods is related to specific radicles combined with unspecific proteins (Landsteiner, 1936). It has, therefore, been suggested that karyogenes determine specificity by forming haptene-like substances which diffuse through the nuclear membrane (Wright, 1941). These specific gene products then combine with unspecific cytoplasmic proteins, that is, karyogene products provide prosthetic groups of specific proteins in cytoplasm. This *modus operandi* provides a consistent explanation of the observed phenomena.

Tissue specificity as determined by transplantation (Little and Gorer, 1943) and serological (Irwin and Cumley, 1943 ; Irwin, 1946) methods is controlled by the nucleus. However, protoplasmic specificity can reside in the cytoplasm as, for example, in mammalian erythrocytes. Not all the prosthetic groups of specific cytoplasmic proteins are of nuclear origin since certain vitamins are of importance in this connection. Proteins necessary for intranuclear functions are presumably formed by components of the nucleus. Euchromatin may perhaps be responsible for the production of specific prosthetic substances while heterochromatin may form unspecific proteins.

8. THE INHERITANCE OF CELLULAR DIFFERENTIATION.

During the development of an animal successive generations of somatic cells exhibit a progressive restriction of potency until the establishment of the final histological types. In mammals these definitive specializations are almost invariably irreversible and mutually exclusive. They may, however, become modified as in despecialized malignant cells. Cellular specialization, apart from some exceptions (Butler and O'Brien, 1942) in lower animals, is usually determinate.

The distinctive type qualities of a lineage of specialized cells are inherited by the daughter cells. In both specialized somatic cells and in whole organisms heredity is manifested as the transmission of specific characters from individual to individual. The persistence of definitive characteristics in the cells of a specialized lineage is independent of immediate localization within an organism

as witnessed by implantation experiments (Strangeways, 1924) and the occurrence of modulation instead of pluripotent dedifferentiation in cells cultured *in vitro*. Somatic inheritance is, therefore, regulated by innate cellular factors.

There are various possible mechanisms whereby the inheritance of cellular specialization and differentiation might be brought about. The hypothesis of differential segregation of nuclear determinants appears to be untenable in view of the equivalent partitions of chromosomal material during mitosis. Yet karyogenes are known to control characteristics of somatic cells. Cytoplasmic determinants are of considerable importance in organismic heredity and the nuclear genotype is unable to control certain formed plasmagenes although it may control their inception. It has been suggested (Rhoades, 1943 ; Sonneborn, 1943) that a differential sorting out of plasmagenes during cell division might account for cellular differentiation. However, in view of the absence of any known mechanism capable of bringing this about it is unlikely that different kinds of plasmagenes could be quantitatively segregated in this way.

Cellular differentiation is a cytoplasmic phenomenon and the most generally accepted view (Child, 1940 ; Harrison, 1940 ; Jennings, 1940 ; Wright, 1941) is that it is inherited through cytoplasmic determinants. If the inheritance of cell specialization is proximately determined by karyogenes, the genotypes of different types of cells in an organism should differ and nuclear material should be equivalently distributed between the daughter cells of a specialized intermitotic cell. The available evidence contra-indicates the first of these requirements and the occurrence of amitosis in certain kinds of specialized cells (Bast, 1921 ; Berrill, 1935, 1943 ; Charipper and Dawson, 1928 ; Elliott, 1936) militates against the second. Further, the chromosomal aberrations of established tumours do not alter the basic cell type. It appears that the inheritance of definitive specialization depends not on the nucleus but on the cytoplasm.

The view that cell specialization and differentiation are regulated by cytoplasm is strengthened by the fact that some plasmagenes suppress the manifestations of others. Darlington (1944) and Haddow (1944) have drawn attention to similarities in the properties of viruses and plasmagenes including that of the possession of suppressiveness. The microsomes of animal cells closely resemble some viruses except in pathogenicity ; at least some viruses and microsomes carry enzymes and can, therefore, modify cell metabolism.

In any one instance intracellular processes must be regulated in some way and selected from a number of possible types to the exclusion of others. Environmental factors modify cell processes and could have differential effects on plasmagenes. The primary differentiation of an egg cell and the subsequent localizations of cells in particular environments (internal and external to the organism) can account for cellular differentiation (Jennings, 1940). Although it appears that the inheritance of definitive specialization does not depend on the nucleus, karyogenes must play a vital role in the initiation of specialized qualities. Inheritance between organisms and the cells of an organism are obviously inter-related ; the expression of the former depends upon the latter and karyogenes fundamentally control both processes.

The present interpretation of these two kinds of inheritance may be summarized as follows. Under the influence of environmental factors the specific

substances produced by nuclear genes derived from the parents are differentially utilized by the cytoplasm of the different kinds of cells of an organism. The existing constitution of the cytoplasm determines the fates of the nuclear inductors and decides the potencies of the cell and its progeny. During development the fates of particular cell lineages are progressively restricted until, in the adult, cytoplasmic constitution is fixed for most kinds of cells. Karyogenes transmitted by the gametes determine the availability of specific substances but the cytoplasm controls the particular uses of these substances.

The differentiation of a specialized type of cell is determined whether it is expressed or not, as in modulated cells, and is inherited through and manifested in the cytoplasm. Dedifferentiation also is obviously a cytoplasmic and not a nuclear phenomenon apart from the provision of essential karyogene products. It is of fundamental and central importance for an adequate understanding of the nature of malignancy (Haddow, 1944). The operative factors in malignancy, therefore, appear to be inherent in cytoplasm and antecedent nuclear factors when present—it is unnecessary to invoke their invariable occurrence—operate through the cytoplasm. This conclusion appears to be in harmony with the available data.

9. THE STRUCTURE OF PROTOPLASM.

The only essential living component of cytoplasm is the hyaline ground substance, hyaloplasm or spongioplasm, and its associated ectoplast. Extraneous membranes and intracellular inclusions can be removed without preventing the continued life and reproduction of the cell (Chambers, 1940, 1943). Living spongioplasm appears structurally homogeneous by microscopic observation and its biological and biochemical properties must be based on submicroscopical differentiations.

The many theoretical contributions to the problem of the submicroscopic structure of protoplasm have been of fundamental importance in that they have shown that the observed distinctive attributes of protoplasm are explicable on the assumption that its morphological basis consists of a complex of fibrillar protein units. The viscosity changes and the associated sol-gel reversals, the properties of elasticity, contractility, thread formation, structural rigidity and cohesiveness are all listed by Seifriz (1936, 1942, 1944) as dependent on the presence of elongated protein particles in the living spongioplasmic matrix. The visible emulsion of protoplasm does not account for, for example, such characteristics as imbibition, elasticity, and contractility (Seifriz, 1942, 1944).

The presence of protein microfibrils in protoplasm has been experimentally verified by anomalous viscosity (Pfeiffer, 1936), true double refraction (Schmidt, 1937), X-ray diffraction (Frey-Wyssling, 1938), electron microscope (Schmitt *et al.*, 1943), and other studies. The sol-gel transformations of living protoplasm depend on the presence of microfibrillæ (Bensley, 1943; Fauré-Fremiet, 1930). The structural organization of cytoplasm is known to depend on fibrous proteins, some of which have been isolated (Banga and Szent-Györgyi, 1940; Bensley, 1938, 1943; Bensley and Hoerr, 1934; Hoerr, 1943; Mirsky, 1936).

The evidence relating to the structural arrangement of these fibrils in living cells indicates that they form a three-dimensional reticulum. The investigations of Mirsky (1936) and the complementary observations of Moore and Miller (1937) have shown that such a structural pattern exists in certain egg cells. In view of the solubility of certain structural proteins in saline solutions the early observations of Chambers and Reznikoff (1928) also provide evidence of the existence of a protein network in *Amœba*. The intermittent displacement of minute crystals in centrifuged amœbæ (Harvey and Marsland, 1932), the results of ultra-centrifugation in *Ascaris* eggs (Beams and King, 1936), the absence of a Devaux effect and also of a "protein error" in the colorimetric determination of pH in living cytoplasm (Chambers, 1940), the tolerance of amœbæ to injections of solutions of picric acid which coagulate proteins *in vitro*, and ultra-violet absorption studies on living protoplasm (Vlès and Gex, 1934) furnish further evidence for a reticular arrangement of the structural protein units. Elasticity and certain other physical properties of protoplasm are shared by non-living gels. In the latter these qualities depend on the presence of interlinked elongated particles (Moyer, 1942). In view of such considerations and data it is generally agreed by protoplasmologists that the submicroscopic structure of living protoplasm is maintained by a network of interlinked linear protein particles. The name "cytoskeleton" has been given by R. A. Peters to this framework.

A second class of spongoplasmic components has been identified and isolated. These are the microsomes or submicroscopic particulates isolated by Claude (1938) from chick embryo tissue and subsequently found in a variety of mammalian tissues. The particulates contain nucleoprotein and lipid, including acetalphospholipoid (Claude, 1940). Since the particulates are known to contain various enzymes, including phosphatase, catalase, succinic dehydrogenase and the cytochrome oxidase system, as well as flavoproteins, lecithin, sterols, and vitamin A (Bensley, 1943), it has been suggested that they function as sites of enzymatic localization in protoplasm (Stern, 1943).

The modern conception of the constitution of living protoplasm is that it comprises three distinct kinds of components. Its structural organization is due to a dynamic, three-dimensional framework of linear protein micellæ, while the ascertained localization of enzymes in discrete particulates accounts for its biochemical heterogeneity. The third component is the continuous aqueous phase containing various solutes which interpenetrates the fibrillar cytoskeleton and bathes the particulates. All three components have been proved to exist and make possible a comprehensive explanation of the salient biological, biophysical, and biochemical properties of protoplasm. The viscosity changes, sol-gel reversals, the properties of elasticity, contractility, thread formation, birefringence, structural rigidity and cohesiveness are obviously explicable on the basis of the structural proteins. The collateral and harmonious existence of numerous enzyme systems, isolated of necessity *in vivo*, is explained by the presence of the particulates and the structural framework. The continuous aqueous phase, which contains components of some enzyme systems (Lazarow, 1943), provides a means of supplying new materials and reactants to all regions of the spongoplasm.

10. THE CYTOSKELETON.

Most students of cell structure accept the view that the ultra-structure of protoplasm is based on linear molecules of protein which form networks (Bensley, 1949). In view of the ease with which reversible sol-gel changes occur in living cells and the immense differences in the viscosity of protoplasm, the conception of a static cytoskeleton is inadequate for many cells. Some at least of the cross-linkages must be readily broken and reformed in such instances. In fully differentiated cells, the cytoplasm of which is a rigid gel (Chambers and Renyi, 1925; Renyi, 1931; Speidel, 1938), the cytoskeletal linkages may be much more permanent and the configuration of the network relatively fixed. Residual valences, van der Waal's forces, salt linkages, and sulphur bridges may play roles in the formations of linkages between the cytoskeletal components.

In the present state of knowledge it can only be stated that a first approximation to the essential nature and characteristics of the cytoskeleton has been attained. The unitary components of the cytoskeleton may be simple fibrillæ of polypeptide chains or they may consist of aggregations of linear protein molecules. In the more recent conceptions of the cytoskeleton interconnections between the component micelles are visualized as consisting of fine fibrils, that is, of polypeptide chains (Seifriz, 1942, 1944).

Discrete cytoskeletal components may be structurally orientated by various physical forces, for example, those resulting from ionic atmospheres. This phenomenon would be of importance in fluid moving protoplasm. Hydrogen bonds and chemical bridges probably serve to connect the components in the gel state. The fundamental importance of disulphide bridges for normal co-ordination of the cytoskeleton and in the etiology of malignancy will be discussed in a later paper. The movements of protoplasm during cell division and sol-gel changes point to an essential similarity of the individual cytoskeletal components in an individual cell, that is, the cytoskeletal pattern is primary and the components interchangeable. As between different kinds of cells of an organism the components may differ. Both the nature of the components and the configuration of the cytoskeleton presumably play an important role in the organization of a cell but the former are probably more important for specialized attributes and the latter for the necessary basic morphology.

The cytological differentiations to which most attention is usually paid are those of use in the classification of cells into histological types. In a consideration of cell organization, however, the relation of a particular kind of differentiation to living protoplasm is of great importance. Simple metaplastic differentiations such as fat droplets or the matrices of bone and cartilage are less directly related to spongioplasm than, for example, myofibrillæ. Myosin is both an enzyme complex (Banga *et al.*, 1947) and a contractile fibrous protein and is also akin to certain structural proteins of cells. Metaplastic differentiations, provided that the synthesis of the necessary substances is possible, are much less dependent on normal organization of spongioplasm than are differentiations directly related to the latter.

The persistence of cell differentiation in an individual cell and its inheritance by specialized cells predicates a uniform organization for each kind of cell.

That is, the spongioplasmic organization in cells of a given type is constant and differs from that in other kinds of cells. This invariant submicroscopic organization of living protoplasm will include chemical and physical as well as structural qualities. The morphological organization of spongioplasm depends upon fibrous structural proteins. These latter are collectively, in part or in whole, equivalent to the cytoskeleton which is synonymous with a co-ordinated system of the elongated fibrillæ forming the functional units of the structural proteins. Stability of spongioplasmic structure can only be achieved by a corresponding stability of cytoskeletal configuration. The lowest form of structural organization of the cytoskeleton is that at which there is no constant and definite arrangement of its components. In this event morphological organization will be at a minimum but diverse chemical and physical properties of the components characteristic of each kind of cell can still produce different cell attributes. Specialized cells with inco-ordinated cytoskeleta would share in common a minimum of spongioplasmic organization but would differ in some of the properties of the inco-ordinated cytoskeletal components and in the secondary effects of these properties. Qualities primarily dependent on the submicroscopic particulates and the continuous aqueous phase would be only indirectly affected by cytoskeletal inco-ordination.

The inability of the cytoskeletal components to form and maintain a precise configuration would result in the absence of stable organization of living protoplasm. Cellular differentiation is a consequence of submicroscopic organization so that a failure or disturbance of the latter will tend to be reflected in the former. The degree of resulting dedifferentiation would be correlated with the degree of dependence of the comparable differentiation upon normal spongioplasmic organization. More highly morphologically differentiated cells would in general be more affected than less differentiated ones by inco-ordination of the cytoskeleton.

11. THE CYTOSKELETAL HYPOTHESIS OF MALIGNANCY.

If for any reason the structural co-ordination of the individual components of the cytoskeleton cannot be maintained, the morphological basis of protoplasmic organization would be lost. The destruction of the submicroscopic structural pattern is not incompatible with the existence and functional activity of the vital processes of spongioplasm since only the co-ordinating relations between the skeletal components have been affected while these discrete separated entities can still subserve functions other than that of structural co-ordination. Thus they could still provide a mechanism for the spatial arrangement of enzyme and lipid molecules which may be attached to them. Temporary "disarticulation" of the cytoskeleton must also occur in flowing protoplasm. It is, however, to be expected that some disturbance of normal cellular activities would take place in addition to the salient structural alterations.

The absence of normal co-ordination could be due to intrinsic changes in the components themselves or to extrinsic modifications in the intraspongioplasmic milieu which might be brought about in various ways, for example, through

enzymatic activities of modified submicroscopic particulates or viruses. Whatever the mechanism of inco-ordination might be, certain important consequences would result from its occurrence. Protoplasmic structure is dependent upon the organization of the cytoskeleton and there is an intimate relation between cellular differentiation and cell structure. The destruction of spongioplasmic organization by inhibiting the development of normal cell structure could prevent normal differentiation. Accordingly, an anaplastic condition of cells could, therefore, be induced by a lack of co-ordination between the cytoskeletal components. The chief biological characteristics of malignant cells which are responsible for the pathological effects of cancerous tissue can be derived from the basic condition of dedifferentiation. If this interpretation and that of the dependence of differentiation upon the cytoskeleton are valid, it follows that an irreversible "disarticulation" or loss of co-ordination of the cytoskeletal components can be regarded as an immediate cause of malignancy and that the essential characteristic of some malignant cells is the condition of inco-ordination of the cytoskeletal components. These conclusions can be hypothesized as follows: the persistent loss of normal co-ordination between the components of the cytoskeleton is a proximate cause of the anaplastic state of tumour cells.

12. DISCUSSION.

Neoplasia can be grouped into two major classes, those consisting of normal cells and those consisting of anaplastic cells. Anaplasia or, alternatively, despecialization is a consequence of persistent cellular dedifferentiation which, in this context, denotes the loss by a cell of distinctive qualities characteristic of its normal prototype. However, in order for its loss to result in anaplastic growth a particular quality must be inessential to the life and reproduction of the cell and yet be necessary for the adequate functioning of the cell as a specialized component of an organism.

Cellular dedifferentiation appears to be mainly, but not exclusively, a cytoplasmic phenomenon and may be brought about in various ways. The latter have been discussed briefly elsewhere (Powell, 1948). For the purpose of a preliminary approximation to an interpretation of anaplastic growth a general mechanism equally applicable to different kinds of cells, irrespective of the natures of their individual differentiations, has been considered.

The general conclusion reached is that the fundamental feature of some tumour cells is a disorganization of their living hyaline protoplasm. The cytoskeletal hypothesis itself has been tentatively put forward for consideration since it appears to be in agreement with the established data discussed above. It also provides a common explanation of certain other properties of tumour cells which will be discussed in the following paper together with some general implications of the hypothesis.

13. SUMMARY.

1. It is concluded that cellular differentiation and dedifferentiation are mainly cytoplasmic phenomena.

2. Cellular dedifferentiation is the fundamental characteristic of benign and malignant tumour cells at the cellular level of biological organization.

3. Submicroscopic disorganization of living protoplasm results in cellular dedifferentiation and anaplasia of tumour cells.

4. Inco-ordination of the cytoskeletal framework of the protoplasm of tumour cells can be regarded as a proximate cause of anaplasia and malignancy.

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VI.—THE ORGANIZATION OF THE MALIGNANT CELL.

PART II.—RELATION OF SOME PHYSICAL, MORPHOLOGICAL, AND BIOCHEMICAL CHARACTERISTICS OF TUMOUR CELLS TO THE DISORGANIZATION OF PROTOPLASM.

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1. INTRODUCTION.

THE preceding paper (Powell, 1948) dealt with the induction of a new hypothesis concerning the origin and fundamental nature of anaplastic tumour cells. This hypothesis, that anaplasia is brought about by the persistent disorganization of living protoplasm and especially by the inco-ordination of its ultra-structural protein fibrils, was induced from a consideration of some biological properties of tumour cells which were related to the known structure of protoplasm. In the present paper an attempt is made to show that some other distinctive characteristics of tumour cells are explicable on the basis of this hypothesis even though they were not used in its formulation. These other aspects of tumour cells are best considered separately because they differ considerably in nature although they can be shown to be correlated by means of the hypothesis.

2. PHYSICAL PROPERTIES OF THE PROTOPLASM OF TUMOUR CELLS.

The structural organization of living protoplasm depends on its contained fibrous proteins. Its salient physical properties, like those of non-living colloidal systems of fibrous units, are consequences of the properties of the fibrous components and the continuous medium. The available evidence, especially from studies of polymerization processes, indicates that typical gel structure depends upon cross-linked and elongated structural units. In many cells, such as amoeboid ones, the cytoskeleton is a dynamic structure and its linkages are easily disrupted and re-formed. The individual cytoskeletal components must be structurally interchangeable to at least a very considerable extent. In fluid protoplasm these components are presumably functionally discrete units, while in the gel phase they are interconnected. Micro-dissection studies have shown that the cytoplasm of fully differentiated cells is a gel (Chambers and Renyi, 1925; Renyi, 1931; Speidel, 1938). Mirsky (1936) has shown that the development of a framework of protein fibrils in cytoplasm greatly increases its resiliency and rigidity. The separated elements of a fibrous colloid may be mutually co-ordinated by various physical forces so that some degree of structural organization is possible in a system of this kind.

Some types of protein denaturation are due to the unfolding of polypeptide chains and coagulation to the interaction and aggregation of denatured molecules. Structural microfibrils of protoplasm are "denatured" to some extent in the living state (Mirsky, 1936). A system of discrete unfolded protein molecules is more susceptible to changes in water content and more easily coagulated than one of similar units arranged in a connected network in which the main bodies of the components are held apart.

Protein microfibrils participate in surface structures such as the ectoplast (Danielli, 1942) or protoplasmic surface of a cell and protoplasmic interfaces. The cytoskeletal hypothesis postulates that the fibrous structural protein components of tumour cells, in contradistinction to those of normal cells, are relatively inco-ordinated and disorganized. Some degree of "articulation" of the cytoskeletal components may exist even in malignant cells. If this hypothesis is valid, changes in the physical behaviour and properties of tumour cell protoplasm should be apparent. Such differences would be expected to be relatively slight but a modification of protoplasmic ultra-structure should produce some characteristic changes at the microscopic level. The postulated modification is, however, relatively insignificant in itself as a physical and chemical change even if its consequences may be far-reaching. The reported differences between the protoplasms of normal and tumour cells in morphological qualities, water relations, and viscosity, will now be considered separately on the basis of the hypothesis.

(i) *The Micromorphology of the Protoplasm of Tumour Cells.*

Several investigators have stated that the protoplasm of malignant cells differs morphologically from that of their normal counterparts. The postulated ultra-structural modification of protoplasm in anaplastic cells should affect the formation of interfaces and similar structures which involve the participation of fibrous structural proteins. A decrease in mechanical strength resulting from weakened attachments between the microfibrils in such interfaces would affect the size limits of discontinuous droplets and vacuoles in the protoplasmic matrix. At this morphological level spongioplasm forms the continuous phase and non-living constituents constitute the disperse phase of protoplasm.

The recorded greater granulation and opacity of the protoplasm (Lewis, 1927, 1938, 1939; Ludford, 1942), including both nucleoplasm and cytoplasm, of malignant cells are explicable by an increased size of droplets and vacuoles, especially those of formerly submicroscopic dimensions. The thicker nuclear membrane (Lewis, 1938), loss of power of adhesion by some tumour cells to glass surfaces (Ludford, 1933, 1934), and the incapacity of malignant cells to segregate vital acid dyes (Ludford, 1933, 1942), which Ludford (1942) has related to a possible ultra-structural abnormality of their cytoplasm, are also explicable on a similar basis.

(ii) *The Water Relations of Tumour Cells.*

The removal of water within certain limits from a colloidal system of discrete fibrous units should produce more drastic changes than an equivalent loss from

a system of comparable but interlinked units as there is a greater probability of coagulation and improbability of the restoration of original structural relations, including hydration, in the former. The reversibility of desiccation, as measured by the relation between the amounts of water removed and subsequently replaced, has been shown to differ in normal and tumour cells. Normal tissue has been reported to take up twice as much water after desiccation as tumour cells (Okuneff and Nasarbékowa, 1934). This result confirmed the earlier work of Roffo (1930), who found that normal mature and embryonic cells were much less affected by loss of water than neoplastic cells. The effects of dehydration by hypertonic solutions are more severe in tumour than normal cells according to Auler (1936). Shear (1935) has shown that tumour cells take up water more rapidly and to a greater extent than normal cells and has stressed the role of hydrophilic colloids in the extracellular medium. These results all appear to be explicable on the assumption that the cytoskeletons of tumour cells are looser or less firmly interlinked structures than those of normal cells.

(iii) *The Viscosity of the Protoplasm of Tumour Cells.*

Various workers have found differences between normal and tumour cells in their responses to ultra-centrifugation. Viscosity implies resistance to flow or movement, either of the fluid or colloid itself or of contained constituents. A change in behaviour under centrifugal force indicates an alteration in viscosity. Guyer and Claus (1936-7, 1939) observed that a variety of tumour cells and rat hepatoma cells (Guyer and Claus, 1942) had a greater cytoplasmic viscosity than normal cells. Cowdry and Paletta (1941) studied viscosity changes in the nuclei of mouse epidermal cells treated with methylcholanthrene and found the lowest viscosity values in malignant cells; papilloma cells gave values intermediate between those for the former and for normal cells. Similar results were obtained in studies on human skin cancers while the viscosity of normal cells increased as development and differentiation proceeded.

The resistance of protoplasm to centrifugal force is contingent upon the resiliency of the cytoskeletal framework since the living hyaline matrix forms the continuous phase of protoplasm. A system consisting of a framework of linear units has greater mechanical strength than a system consisting of an equal number of similar but disconnected units contained in an equal volume of fluid medium. However, the viscosity of a colloid due to a certain number of interlinked fibrils could be equalled and exceeded by the viscosity resulting from the presence of larger numbers of similar but discrete fibrils. The viscosity of protoplasm would be expected to be less in cells with disarticulated cytoskeletons and containing the same number of components per unit volume of protoplasm than in comparable cells with normal cytoskeletons. If the free components increase greatly in number, protoplasmic viscosity should also increase to a limit imposed by the attainment of a maximum number of disconnected components per unit volume of protoplasm. If these disconnected units have the power of self-reproduction or if their formation by other organelles is regulated by the development of an organized cytoskeleton, they should increase in number in cells with disarticulated cytoskeletons.

There is, therefore, the possibility of an initial decrease in protoplasmic viscosity during the early stages of carcinogenesis and also a possibility of a subsequent increase. Cytoplasm would be affected before the nucleus by an extrinsic carcinogen. The position would, however, be complicated by the simultaneous presence of normal and abnormal cytoskeletal components in pre-cancerous cells. Carcinogenic agents are known to produce an initial decrease followed by an increase in cytoplasmic viscosity in *Paramecium* (Mottram, 1942). The observed changes in viscosity during carcinogenesis and in fully malignant cells appear to be in agreement with the hypothesis put forward.

3. CYTOLOGICAL ABERRATIONS OF TUMOUR CELLS.

In addition to exhibiting some degree of dedifferentiation tumour cells as a class are distinguished by the presence of certain cytological aberrations although comparable abnormalities have been observed in non-neoplastic cells under both normal and experimental conditions. Such abnormalities are presumably secondary to, or concomitant with, neoplastic processes. Although tumour cells usually differ from their normal prototypes by either the presence or greater abundance of cytological aberrations no ubiquitous and specific morphological characteristic has been found to be restricted to the former. Before considering these aberrations it is desirable to discuss briefly the mechanisms underlying nuclear and cell division.

The reproduction of cells entails two distinct and separable major processes, nuclear division and cytoplasmic cleavage. Disjunction of these two processes occurs naturally and under experimental conditions. A variety of agents, all of which affect the viscosity of protoplasm, bring about dissociation of the division processes (Gray, 1931). Nuclear division without cell division occurs normally in arthropod eggs which pass through a syncytial stage. On the other hand, enucleate fragments of *Arbacia* eggs were induced by Harvey (1936) to cleave repeatedly.

Mitosis is the usual type of nuclear division in somatic cells and amitosis appears to be restricted in mammals to a few kinds of specialized tissues (Berrill, 1935, 1943). In either instance nuclear division is normally followed by cleavage of the cytoplasm which is controlled by rhythmical changes in viscosity. The structures chiefly concerned in the mitotic process, apart from the chromosomes themselves, are the centrosomes, asters, and spindle, but only the last is essential for mitotic division since centrosomes and asters are absent from some cells. According to Conklin (1924) both the asters and spindle are formed partly from nuclear and partly from cytoplasmic material. However, spindles and asters have been observed to arise *de novo* in cytoplasm in the absence of a nucleus.

Asters are elastic and relatively rigid structures (Chambers, 1917, 1924) and their physical properties are shared by experimentally produced monasters and cytasters. It has been shown by Wilson (1901) that monasters can initiate the process of nuclear division which, however, stops short of the anaphasic migration of the daughter chromosomes. Available evidence suggests that asters are usually transient organelles formed anew at the onset of each division cycle.

Like asters the mitotic spindle is an elastic gel and has been proved to have an objective existence by microdissection (Chambers, 1917, 1924 ; Foot and Strobell, 1905) and ultra-centrifugation (Morgan, 1910) studies. Lewis (1923) has shown that spindle fibres can be rendered visible in living cells by acidifying the culture medium and that the phenomenon is reversible. However, the spindle is visible in some untreated living cells (Cooper, 1941). Spindle fibres are birefringent (Schmidt, 1937). The characteristics of both spindle and asters indicate that they are fundamentally composed of elongated protein molecules. A spindle with at least two poles is essential for the separation of chromosomes during anaphase and the poles determine the locations of the daughter nuclei. Chromosomes situated outside the spindle are left lying in the cytoplasm and not incorporated in the nuclei.

In astral cell cleavage the asters and spindle actively participate in this process since alterations in the size and position of these organelles are always associated with corresponding modifications in the cleavage furrows and daughter cells. Observations on dividing cells have revealed that astral cleavage depends on the temporary formation of two centrally placed rigid and elastic spheroids in the cell and the synchronous movement of fluid protoplasm around them (Chambers, 1924 ; Gray, 1931). Although visible asters are not present in disjunctively dividing cells Gray (1931) has put forward a similar explanation of anastral cleavage. On the other hand, some workers, for example, Lewis (1942), have concluded that contraction of the gelled cortical cytoplasm is almost entirely responsible for disjunctive cleavage. Chambers (1938) has shown that both mechanisms play a part in the cleavage of *Arbacia* eggs. However, it is generally accepted that both astral and anastral cleavage are achieved by a series of regular changes in the regional viscosity of the cell. The fundamental process therefore appears to be the temporary differentiation of the cell in respect to the regional segregation and arrangement of those elements of living protoplasm that are responsible for its variable viscosity. These elements are the fibrillar components of the structural protein framework of protoplasm. Cytoplasmic division (Harvey, 1936) and the formation of a spindle (Beams, 1943) are known from ultra-centrifugation experiments to depend on the hyaline matrix of protoplasm. Thus, established data point to the dependence of both nuclear and cytoplasmic division on particular arrangements of linear protein molecules which are largely provided by cytoplasm. During mitosis nuclear and cytoplasmic materials are closely juxtaposed if not intermingled and the ultra-structure of spongioplasm and nucleoplasm appears to be basically similar. Similar or identical microfibrils are present in both and may be interchangeable. These microfibrils are temporarily rearranged during division to bring about the formation of the spindle and asters, viscid astral regions, and surrounding fluid protoplasm, and the contraction of the gelled cortical layer.

Pronounced inco-ordination of the structural microfibrils would, therefore, result in observable aberrations of nuclear and cell division. Malignant cells are well known to be characterized by a high incidence of such abnormalities and Ludford (1942) has stated that *in vitro* cultures of malignant cells can be correctly diagnosed solely on this basis. An attempt will now be made to show that some of the more common cytological abnormalities of tumour cells

are compatible with the hypothesized ultra-structural disorganization of their protoplasm.

(i) *Abnormalities of the Mitotic Spindle and Chromosomes.*

Inco-ordination of the microfibrils taking part in the structure of the spindle would produce structural and functional derangements. Abnormal mitotic figures, including multipolar spindles (Kemp 1930 ; von Moellendorff, 1940) and the absence of a spindle (Ludford, 1930, 1942), have been reported in tumour cells and also in pre-cancerous cells (Block, 1932 ; Mendelsohn, 1935). Derangements of the spindle are accompanied by aberrations of the chromosomes since the anaphasic migration of the latter is governed by the spindle. Aberrations of chromosome segregation, including non-disjunction of metaphase chromosomes and unequal partitions of chromosomes between paired daughter nuclei, result from faulty functioning of the spindle and are of frequent occurrence in malignant cells (Kemp, 1930 ; Levine, 1931 ; Ludford, 1942). Ludford (1930, 1942) has described a great variation in the chromosome numbers of the cells of a single tumour. Levine (1931) and Lewis (1939) have also described abnormal chromosome numbers in malignant cells. Polyploidy, which is common in tumour cells, may also result from failures of the spindle mechanism.

Dissociation of the processes of daughter chromosome separation, dissolution of the nuclear membrane and spindle formation could result in endomitosis which Bieseke *et al.* (1942) has demonstrated to be of widespread occurrence in tumour cells. Endomitosis itself can give rise to polyploid nuclei, polytene chromosomes, and diplochromosomes (Bieseke *et al.*, 1942), all of which Bieseke has found in tumour cells.

(ii) *Binucleate and Multinucleate Cells.*

The formation of binucleate and multinucleate cells is directly traceable to disharmonies between nuclear division and cell cleavage, that is, nuclear division without division of the cytoplasm, such as Levine (1931) observed in tumour cells. Koller (1943) has recorded extra nuclei in the cells of a variety of human tumours and Pullinger (1940) has observed the development of binucleate cells during early epidermal carcinogenesis in mice.

(iii) *Polymorphism.*

The presence of cells representing diverse variations of the parent type has often been observed in individual tumours. As Mottram (1942) has pointed out, this phenomenon of polymorphism is a fundamentally important feature of malignant cells. The degree of polymorphism is highest in a primary tumour, less in metastases, and least in continued serial implantations (McIntosh, 1933). After several successive implantations a relatively homogeneous tumour consisting of one kind of abnormal cell tends to become established. Mottram (1934, 1935, 1936*a* and *b*) has shown that certain primary tumours consist of conglomerations of cell types having different growth rates and that the proportion of cells with the highest division rate increases until such cells are dominant. Since the more anaplastic cells divide more rapidly such tumours become more

anaplastic with continued development. This is in accordance with the clinical observations that secondary growths very often consist of cells that are more malignant than those of the primary tumour.

Chromosomal aberrations of tumour cells modify the genic equipment of affected cells. Some of these cells which lack some chromosomal material will be deprived of the specific products of the missing genes. If the losses of gene material vary from cell to cell of an individual tumour, the affected cells will differ among themselves and from the original neoplastic cells. However, since cellular differentiation is usually irreversible such secondarily modified cells will be less differentiated than the original cells but of the same basic type. Loss of genetic material beyond a certain limit results in cell death but some viable tumours have been reported to lack the normal chromosomal equipment.

Abnormalities of the mitotic spindle itself are common in tumour cells and give rise to variations in chromosome number and arrangement. The latter give rise to polymorphism and can bring about disturbances in the euchromatin—heterochromatin balance which Koller (1943) and Darlington and Thomas (1941) have suggested is the initiating cause of malignancy. Polyploidy, endomitosis and its sequelæ, binucleate and multinucleate cells, are also consequences of abnormal division processes which can be traced back to inco-ordination of the microfibrils of the structural proteins of protoplasm. The inco-ordination of these microfibrils appears to be a permanent feature of the cells of many tumours. The occurrence of comparable cytological abnormalities in non-neoplastic cells is presumably due to temporary inco-ordination of the microfibrils and not due to their intrinsic inability to form co-ordinated structures.

4. THE BIOCHEMISTRY OF MALIGNANT CELLS.

The morphological dedifferentiation of tumour cells can be regarded as the obverse of their biochemical peculiarities. Tumour cells arising from specialized cells share the characteristic of being despecialized. Different kinds of malignant cells, however, differ from each other in properties which are related to the particular specializations of the cells from which they are derived. Greenstein (1943) has stated that the enzymatic activities of diverse mouse tumours are unspecialized in comparison with those of normal tissues. Hepatoma cells are distinguished by the loss of certain highly specialized enzyme functions possessed by normal liver cells (Dickens and Weil-Malherbe, 1943). On the other hand, tumour cells often show a partial retention of specialized biochemical processes.

An ubiquitous characteristic common to different kinds of tumour cells is of greater significance for the understanding of the etiology of malignancy than a quality confined to one given kind of tumour. Most cancer research workers would probably agree that of all the biochemical peculiarities of tumours the power of aerobic glycolysis is outstanding. According to Burk and Winzler (1944) the great majority of tumours are distinguished by their low respiratory quotients and their powers of aerobic and anaerobic glycolysis. In view of this fact and of the complexity of the biochemistry of tumour cells the respiratory and glycolytic enzyme systems have been selected for further consideration.

The properties of living protoplasm predicate biochemical heterogeneity. Such phenomena as the harmonious co-existence of antagonistic or mutually destructive enzyme systems, the efficient synchronization of the components of a complex enzyme system and the orderly regulation of cell metabolism depend upon precise ultra-structural arrangements of the functional enzyme units in living protoplasm. Gross autolysis is probably the best known example of the effects of a breakdown of cellular organization. However, lesser disorganizations of the ultra-structure of protoplasm have pronounced effects on biochemical processes. Thus the Harden-Young diphosphate-hexose ester accumulates in muscle extract but occurs in only insignificant amounts in intact muscle (Needham, 1932). Cytolysis inactivates dehydrogenases in bacteria, although cytochrome oxidase is not appreciably affected, and respiration is greatly reduced (Quastel, 1932).

The respiratory and glycolytic enzyme systems are known to be intimately associated with cell structure (Korr, 1939) and their components are micro-morphologically arranged in spongioplasm. Growth and differentiation have been shown in many instances to depend on the functional activity of the cytochrome-cytochrome oxidase system even when the basal metabolism is carried on by other means. There is a close inter-relationship between the Warburg-Keilin system, cell differentiation and the structure of protoplasm. Detailed information on these inter-relationships is available for *Melanoplus* and echinoderm eggs.

The development of *Melanoplus* is interrupted by a diapause during which there is complete cessation of cell division and differentiation (Slifer, 1931) and greatly reduced respiration (Bodine, 1934). Bodine (1934) and Bodine and Boell (1934) found that CO and CN inhibit the respiration of non-diapause embryos but do not affect that of ones in diapause. Similarly, physical disorganization diminished the respiration of non-diapause embryos but had no effect on the oxygen uptake of diapause embryos (Bodine and Boell, 1936). The addition of paraphenylenediamine increases the respiration of brei prepared from non-diapause embryos and this increase is sensitive to cyanide. It was, therefore, concluded by Bodine and Boell that the cytochrome-cytochrome oxidase system is sidetracked during diapause, when differentiation is at a standstill and basal metabolism carried on by other means. It was also demonstrated that disorganization of protoplasm decreases respiration although the individual components of the respiratory enzyme system are present. This point has been confirmed by ultra-centrifugation studies on the same organism (Bodine and Boell, 1936) and also for *Ascaris* embryos (Huff and Boell, 1936). Unlike diapause embryos of *Melanoplus* the non-diapause embryos showed decreased respiration after centrifugation.

Cytolysis greatly reduces the respiration of echinoderm eggs, and anærobiosis halts their development (Harvey, 1930). As in many other groups of animals, fertilization of sea-urchin eggs is rapidly followed by an increased oxygen uptake (Rothschild, 1946). Cyanide decreases the respiration of fertilized eggs but not that of unfertilized ones (Runnström, 1930). The addition of dimethylparaphenylenediamine greatly increases the respiration of both kinds of eggs; cyanide now inhibits the respiration equally (Runnström, 1930). Although

methylen blue increases the respiration of both fertilized and unfertilized eggs, the increase is greater in the latter (Runnström, 1932). This increase is unaffected by cyanide. Dehydrogenases were found to be present in both kinds of eggs to the same extent. Runström (1930, 1932) concluded from these experiments that the components of the Warburg-Keilin system are present in both fertilized and unfertilized eggs but that in the latter these components are isolated from one another and that fertilization brings about the functional co-ordination of the units of the enzyme system by a reorganization of the protoplasm.

These results and conclusions gain greater significance when related to the work of Mirsky (1936) and Moore and Miller (1937) who have shown that fertilization of sea-urchin eggs initiates a reorganization of the cytoplasm which involves a rearrangement of the structural protein microfibrils. Thus it appears that the cytochrome-cytochrome oxidase system is intimately associated with the submicroscopic structure of spongioplasm and functionally dependent upon a precise co-ordination of microstructural components. Shapiro (1935) has shown that the characteristic retardation of respiration in sea-urchin eggs before fertilization and the subsequent increase are associated with the hyaline protoplasm of the egg.

The addition of pyocyanine increases the respiration of unfertilized sea-urchin eggs more than that of fertilized ones; this pigment also stimulates the respiration of egg brei (Runnström, 1935). The inhibition of the respiration of fertilized eggs by cyanide is counteracted by pyocyanine but the cessation of cell division and development still persists (Korr, 1937). "Ferrous" respiration is, therefore, intimately linked with cell differentiation but the latter is not limited through the provision of available energy. Korr's researches (Korr, 1937) on the temperature coefficients of ferrous and non-ferrous respiration have shown that the mechanisms for the transfer of activated hydrogen from the substrates to cytochrome are the limiting factors in unfertilized sea-urchin eggs.

Treatment of living cells with cyanide has long been known to decrease respiration and simultaneously accelerate glycolysis. Carbon monoxide has similar effects. Glycolysis is promoted by mechanical injury, lowered oxygen tension or damage to the oxidative enzymes (Fleischmann, 1939; Kempner, 1939). If the cytochrome-cytochrome oxidase system is impaired, the Pasteur effect is inhibited and aerobic glycolysis promoted. The abolition of the normal organization of respiring protoplasm can, therefore, concurrently decrease respiration and increase aerobic glycolysis.

There is definite evidence that the Warburg-Keilin system is not fully functional in malignant cells since it can be by-passed by reversibly oxidizable dyestuffs which on addition to the cells greatly increase the consumption of oxygen and inhibit aerobic glycolysis (Barron, 1930; Friedheim, 1934). Most normal tissues show comparable responses only after previous injury (Barron, 1930). The metabolism of tumour cells has, therefore, been compared with that of injured normal ones. These phenomena collectively indicate that in malignant cells the components of the Warburg-Keilin enzyme system are in some way not normally integrated with one another to form an efficient respiratory chain as they are in normal cells. Accordingly, disorganization of the sub-

microscopic structure of protoplasm of malignant cells could account for these particular metabolic peculiarities.

Diverse mouse and rat tumours are respectively much more alike in their enzymatic activities than are comparable normal tissues (Greenstein, 1945). A disorganization of protoplasm would be expected to affect many other enzyme systems in addition to the glycolytic and respiratory ones, especially those associated with cell structure and comprising several components. Some of the more characteristic enzyme peculiarities of tumour cells can be explained by reference to thiol groups which are widely suspected to be concerned in some way with carcinogenesis and the typical properties of tumour cells.

Disulphide bridges are one of the more important kinds of linkage between linear protein chains and are present in the structural proteins of protoplasm. The observations of Fauré-Fremiet (1934*a* and *b*) that protoplasm in the sol state gives a positive nitroprusside reaction whereas gelled protoplasm gives a negative result suggests that the disulphide linkages of connected structural microfibrils are replaced by sulphhydryl groups in unconnected ones. Disconnected fibrils would expose thiol groups in excess over the normal range; the same result would be more permanently achieved by an asymmetrical destruction of thiol groups on the protein fibrils. The oxidation-reduction potential of the intraprotoplasmic milieu would consequently be shifted towards the negative side. It is known (Stern and Willheim, 1943) that living tumour cells have a marked negative potential.

Sulphydryl groups are essential for the activity of many enzymes; they stimulate the activities of some and inhibit the activities of others. Tumour cells typically have considerable proteolytic powers (Maver and Thompson, 1946; Stern and Willheim, 1943) and proteolytic enzymes are stimulated by thiol-containing substances (Zamecnik and Stephenson, 1946). Greenstein (1943, 1945) has shown that catalase is greatly reduced in tumours, and Marks (1936) has suggested that thiol substances such as glutathione are important in inactivating this enzyme. The reduced activity of arginase in tumour cells (Greenstein, 1943, 1945) may also be attributable to the fact that it is inhibited by thiol donors (Klein and Zeise, 1933). Similarly, glycolysis is stimulated by thiol-containing substances some of which simultaneously reduce respiration (Lipmann, 1942; Quastel and Wheatley, 1932). According to Kempner (1939) leucocytes show high glycolytic activity. This characteristic may be associated with the fact that their cytoplasm is frequently in the sol phase because of their amoeboid movements. It is also pertinent in this context that the energy expended by dividing cells appears to be derived from glycolysis, since the division processes involve regional sol-gel changes. Sulphydryl groups of the structural proteins are probably concerned in carcinogenesis since many carcinogenic agents are known to react with such groups and chemical carcinogenesis can be modified by thiol donors or inactivators (Crabtree, 1941; Reimann and Hall, 1936).

5. DISCUSSION.

The previous paper (Powell, 1948) described the induction of the hypothesis that submicroscopic disorganization of living protoplasm can bring about the

malignant state in affected cells. This hypothesis was derived from a consideration of the biological qualities of tumour cells and protoplasmic structure. It has been shown in the present paper that various other properties of tumour cells can also be explained on the basis of this hypothesis although these qualities were not utilized in its original formulation. These other properties are in fact implicit in the logical content of the hypothesis.

Neoplastic growths may be classified in two broad categories, those which develop in direct consequence of derangements of the organization mechanisms of the affected organisms and those which result from intrinsic modifications in the component cells of the growth. Neoplasms belonging to the first category include terata, anidian embryos, and possibly certain abnormal amphibian embryos. In these instances unorganized growth results from disturbances in the mechanisms of organization such as the presence of supernumerary organizers or the absence of normal organizers. The component cells of these abnormal growths appear to be normal and not anaplastic. On the other hand, the cells of anaplastic neoplasia, which comprise the vast majority of tumours, are despecialized and innately modified. Such cells lack the power of completely responding to organization mechanisms and the extent of this disability is correlated with the biological grade of the neoplasm.

Anaplastic neoplasia may be subclassified on the basis of the histological potencies of their parent cells. They may arise from relatively unspecialized and pluripotent cells or from specialized cells. Tumours of the first kind are histologically heterogeneous and those of the second kind are homogeneous. The biological grade of the tumour is not primarily determined by the potency of the original parent tissue but is dependent upon the extent of the despecialization present in the derived cells. The different grades of anaplastic tumours can be arranged in a sequence of increasing disorganization both of their component cells and of their "host" organisms. The degree of despecialization and hence the grade of neoplasm depend on the extent of the operative dedifferentiation in each instance. The biological behaviour of anaplastic cells is characteristic for each grade of tumour irrespective of differences in the nature of the dedifferentiation in particular instances.

Despecialization, whether it is due to incomplete or to retrogressive specialization, is brought about by cellular dedifferentiation. The causes of dedifferentiation are multiple. Dedifferentiation may be caused by the loss or dysfunction of specific substances necessary for normal differentiation which could result, for example, from mutations of nuclear genes or plasmagenes, the effects of extrinsic viruses or modifications of cellular substrata. Tumours arising in these ways would differ among themselves if the modified specific cellular sites vary—as is almost inevitable.

In this preliminary survey of the phenomena of neoplastic growth an attempt has been made to correlate the attributes shared by the great majority of tumours and to ascertain whether these attributes could be traced to a common factor. These general characteristics include morphological and biochemical dedifferentiation, especially the typical respiratory and glycolytic metabolism, and cytological aberrations in addition to the biological behaviour of tumour cells. Observed modifications in the physical properties and texture of the protoplasm

of tumour cells have also been utilized in this analysis although relevant data were available for only a few kinds of tumours. Apart from causation by dysfunction of diverse specific cellular constituents, cellular dedifferentiation may also arise from a general unspecific disorganization of protoplasm. Anaplastic cells arising in this way should share many characteristics in common which should be superimposed on their often dissimilar modified specialized properties.

In the present papers greatest consideration has been given to the production of dedifferentiation by disorganization of protoplasm and it has been demonstrated that the attributes of tumour cells listed above can be traced to this common origin. The general thesis put forward is that the essential and fundamental feature of this class of anaplastic tumours is the disorganization of their living hyaline protoplasm. This disorganization occurs at such levels that the modified cells remain viable and reproduce but are unable to co-operate fully with normal cells in the development and maintenance of the organization of an organism.

Since the structural organization of living protoplasm is dependent upon orderly configurations of protein fibrillæ or micellæ, greatest stress has been placed on the importance of the structural proteins collectively comprising the cytoskeleton for an understanding of anaplastic growth. However, the structural proteins are concerned in the chemical organization of protoplasm and all classes of spongioplasmic components are geared together and affect one another. The main conclusion reached is that an irreversible or long persisting disorganization of protoplasm and especially the inco-ordination of the components of the cytoskeletal framework results in an anaplastic transformation of the affected cells. It is possible that the majority of tumours arise in this way.

In such tumours the initiation of the disorganization or inco-ordination may be regarded as the proximate cause of the anaplastic state since it can produce most of the main features of tumour cells and is the root cause of these properties which together constitute the facies of the anaplastic state. Protoplasmic disorganization or cytoskeletal inco-ordination need not necessarily be the primary cause of anaplastic growth but could be brought about by, for example, viruses or gene mutations. On the other hand, inco-ordination of the protein microfibrils may be both a primary and a proximate intracellular cause in many instances.

In view of the diversity of carcinogenic agents, which also have other effects on cells, and the complexity of protoplasm it seems that the competence to respond to such agents by the development of the anaplastic state is determined by a common effect on some ubiquitous substratum in protoplasm. There is abundant evidence that carcinogenic agents affect the fibrous proteins of protoplasm; for example, Mottram (1942) observed that carcinogens change the viscosity of cytoplasm, and cytological aberrations consequent upon alterations in viscosity occur in cells within a few hours of treatment (Pullinger, 1940). Summation experiments with different carcinogenic agents indicate that a common cellular substratum is involved in carcinogenesis.

Plasmosin, the fibrous structural protein obtained from cytoplasm by Bensley (1938) and Hoerr (1948), might in virtue of its nucleoprotein nature be autocatalytic and also form other structural proteins. It is therefore possible that

it is the substratum involved in carcinogenesis. An asymmetrical destruction of members of paired thiol groups which form interfibrillar disulphide bridges would suffice to initiate carcinogenesis. Certain chemical carcinogens have a remarkable affinity for purine constituents of nucleoproteins and many kinds of carcinogens are known to affect thiol groups. On the other hand, the activities of modified submicroscopic particulates, which are known to carry enzymes and contain lipid capable of taking up carcinogenic hydrocarbons and ribosenucleoprotein, and of the possibly related carcinogenic viruses, may produce an inco-ordination of the structural elements of protoplasm.

The long latency period in the development of malignancy in some instances might be due to the gradual accumulation of abnormal fibrils. The cytoskeletal hypothesis also provides an explanation of the existence of different grades of tumours since varying degrees of inco-ordination of the structural components and protoplasmic disorganization would produce correlated variations in the extent of cellular dedifferentiation. Furthermore, since cellular specialization is usually an irreversible process, the hypothesis also explains the similarities between comparable tumours and between tumours and their parent tissues. The disorganization is superposed upon the differentiated protoplasm of histologically different cells so that tumour cells are alike in being disorganized but differ in their kinds of despecialization. Anaplastic tumours can be regarded as disorganized growths and other neoplasia as unorganized growths.

Many of the issues raised in these preliminary papers require further and detailed consideration since it has been necessary to confine attention to general principles for the most part. The main thesis and subsidiary ones have been generalized and over-simplified. However, it was desirable to place the general pattern of data in relief, and it is the correlation of the most important phenomena of tumour growth by means of the tentative hypothesis put forward that is of most significance. The salient contribution made is possibly the demonstration that a single basic hypothesis can provide an explanation of diverse aspects of tumour growth which have hitherto remained mutually isolated phenomena and not been integrated into a single unified concept of anaplastic growth. Apart from the colligation of such characteristics of tumour cells the hypothesis may be of practical value since it suggests that the anaplastic state might be counteracted by substances which cross-link protein fibrils, for example, in the manner suggested elsewhere (Powell, 1944). The formation of an "artificially" cross-linked cytoskeleton should neutralize the malignant state. Such considerations provide the basis for a new approach to the chemotherapy of tumours especially since it has been shown that certain substances with the specified properties inhibit tumour growth in mice (Powell, 1944).

6. SUMMARY.

1. Certain distinctive physical, cytological and biochemical attributes of tumour cells are discussed.
2. The hypothesized submicroscopical disorganization of the protoplasm of tumour cells is shown to provide a common explanation of these characteristics.
3. The general significance of the hypothesis in relation to neoplastic growth is briefly discussed.

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VII.—PRELIMINARY STUDIES IN THE ELECTRON MICROSCOPE OF SOME PLANT VIRUS INCLUSION BODIES.

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ONE PLATE.

INTRODUCTION.

It has long been known that some virus diseases cause abnormal inclusion bodies to be produced in the cells of the hosts. Ever since Iwanowski (1903) first described them in plants infected with tobacco mosaic virus arguments have raged about their origin and nature. All contain protein and some have been shown to be infective and therefore to contain virus (Sheffield, 1939, 1941). The preliminary electron microscope studies to be described here have confirmed this and also shown the structure of the smaller constituents of some of the inclusions.

METHODS.

Inclusions were isolated from the cells of leaf hairs as these had been found to be the easiest to manipulate. The micro-dissection methods used were substantially similar to those already described (Sheffield, 1939, 1941).

Before starting the dissections the approximate centre was marked on a number of electron microscope specimen grids by slightly enlarging one hole in each with the aid of a blunt needle. The grids were then cleaned, dried, and a collodion membrane was picked up on each using the technique described by Crook and Sheffield (1946). Each was placed in the centre of a clean microscope slide and stored in a covered Petri dish until needed.

The inclusions were too large to be examined intact in the electron microscope so where possible the contents were dispersed in water. This was done by picking them up in a pipette containing water. The pressure within the pipette was so adjusted as to hold the drop of suspension near the tip. Dissections were done from below the specimen, but the placing of material on the specimen grid can be more easily done from above. As soon as the material to be examined was held in the pipette, the moist chamber was removed from the microscope stage and the high-power objective used for the dissections was replaced by a $\frac{2}{3}$ inch and the pipette was rotated through approximately 180°. A slide bearing a collodion-coated grid was placed on the microscope stage and the enlarged hole brought into the centre of the field. The pipette tip was brought over this hole and lowered until almost in contact with the membrane

and the pressure within the pipette was slowly increased so that a drop was ejected from the tip. This drop was rapidly touched down on to the collodion membrane and dried within a few seconds. At this stage the preparations were usually ready for examination. However, it was sometimes necessary to remove inorganic salts, especially if these had been added in the course of the dissections, in which case specimens were washed several times in distilled water. This was done either by flooding and removing the water by touching the edge of the drop against filter paper, by immersing several times or by floating with the membrane downwards on the surface of cleaned distilled water.

Electron micrograms were made by the methods described by Crook and Sheffield (1946).

RESULTS.

1. *Healthy Cell Contents.*

A micropipette containing water was inserted into healthy hair cells of tobacco, tomato, or *Solanum nodiflorum* and material was withdrawn, no attempt being made to separate cytoplasm from cell sap. Some preparations were washed to remove inorganic salts. Electron micrograms of this mixture of sap and cytoplasm consisted chiefly of unidentified material varying in particle size from about 20μ to beyond the limits of resolution of the electron microscope (fig. 1). A few mitochondria were found and very occasionally a crystal was seen. The crystals were rhombic with sides rarely exceeding 0.5μ and they were even less common in young plants than in plants about 2 months old.

2. *Tobacco Mosaic Virus Strains.*

Electron micrograms of purified preparations of tobacco mosaic virus show rod-shaped particles of 15μ diameter. Some workers have argued that these rods are of constant length (Oster and Stanley, 1946) but workers in this laboratory find that the rods vary from a length not exceeding the diameter to several microns according to the treatment received (Bawden and Pirie, 1945; Crook and Sheffield, 1946). The optical microscope has shown that this virus causes a number of morphologically dissimilar types of inclusion body. So far only two types, one amorphous and one crystalline, have been examined. Preparations made from tobacco, tomato and *S. nodiflorum* gave similar results in both cases.

(a) *The Amorphous Body.*—The amorphous bodies caused by the aucuba strain are formed by the aggregation of granules. As well as protein, they contain mitochondria and fats (Sheffield, 1931). At a late stage, they may give rise to protein crystals of various shapes: those most usually formed are hexagonal (Kassanis and Sheffield, 1941). The amorphous bodies are fragile and disintegrate at once if pressure is exerted on the cell wall near them or if they are stabbed. They can, however, be isolated if taken out into a solution isotonic to the cell contents (Sheffield, 1939). Dissections were done in $0.1\text{ M. }(\text{NH}_4)_2\text{SO}_4$. Bodies were extracted into this solution and washed in several changes of it. Finally they were picked up into a drop of water contained in a



Fig. 1.

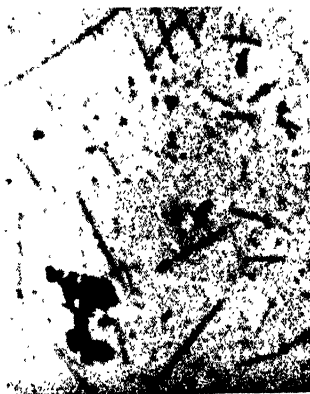


Fig. 2.



Fig. 3.

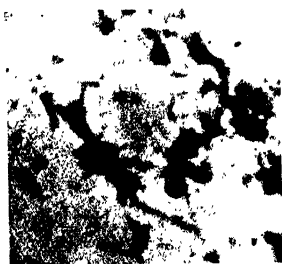


Fig. 4.

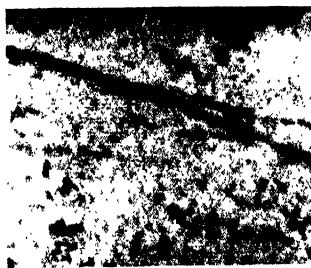


Fig. 5.



Fig. 6.



Fig. 7.



Fig. 8.



Fig. 9.

micropipette where, as a result of the reduced osmotic pressure, they disintegrated and formed a suspension. Drops of this suspension were placed on collodion membranes in the usual way. All preparations had to be washed to remove the crystals of $(\text{NH}_4)_2\text{SO}_4$.

Electron micrograms of the contents of these inclusions are like those of the healthy cell contents in that they contain large quantities of small particles of unidentified material but they also contain rods. These rods, like those of the purified virus preparations, are of constant width but they vary in length, and they are almost certainly the virus. Some of the small round particles seen in the micrograms may also be virus for it would be impossible to distinguish between some of the healthy cell contents and the smallest particles seen in purified preparations of tobacco mosaic virus (fig. 2 and Crook and Sheffield, 1946).

(b) *Hexagonal Crystals*.—These give protein reactions only and are produced by several strains of tobacco mosaic virus. They may arise by crystallization of the amorphous body just described or directly from cell contents which in the optical microscope are otherwise indistinguishable from those of healthy cells (Kassanis and Sheffield, 1941). They are even less stable than the amorphous inclusions and merely puncturing the wall of the cell containing them causes them to break at once to give long narrow needle-like fibres. It was possible to remove this mass of fibres from the cell by keeping it enclosed in a sheath of cytoplasm, but directly any attempt was made to remove the cytoplasm the mass of fibres fell apart and fibres became mixed with particles of the enveloping cytoplasm. No attempt was therefore made to test the infectivity of the crystals nor was it possible to ensure that suspensions made from these crystals for examination in the electron microscope were entirely free from cytoplasm. Crystals were extracted into 0.1 M. $(\text{NH}_4)_2\text{SO}_4$, placed on collodion membranes, dried and then washed several times in distilled water to remove the salt.

Electron micrograms were similar to those obtained from the amorphous inclusion bodies. They contain rods of constant width but varying length which are presumably tobacco mosaic virus and also small unidentified particles some of which may be virus (fig. 3).

(c) *Other Cell Contents*.—Other types of amorphous and fibrous bodies associated with tobacco mosaic virus have not yet been examined in the electron microscope. But material has been taken from infected cells, which showed no abnormal inclusions in the light microscope, and also from those parts of inclusion-containing cells which were remote from the inclusion. No attempt was made to separate the cytoplasm from the cell sap. Cell contents were extracted either into water or into 0.1 M. $(\text{NH}_4)_2\text{SO}_4$. Micrograms were similar to those obtained from the inclusion bodies but the amount of solid matter was considerably less.

3. *Severe Etch Virus*.

Some attempts to isolate this virus resulted in solutions which reacted with anti-serum up to dilutions of 1/512 (Bawden and Kassanis, 1941). The solutions

showed anisotropy of flow suggesting that the virus particles are rod-shaped but unfortunately these preparations were not examined in the electron microscope. However, some partially purified preparations made by spinning alternately at 8000 and 40,000 r.p.m., and which did not show anisotropy of flow were found to contain rods (fig. 4). These preparations were kindly made by Dr. E. M. Crook. The virus is of particular interest cytologically for it is one of the only two plant viruses known to cause inclusions in the host nuclei (Kassanis, 1939). Large amorphous inclusions are also produced in the cytoplasm of many of the cells.

(a) *Nuclear Inclusions*.—These take the form of rectangular plates which gradually increase in size until their sides may reach a length of 10μ . They are usually about 0.5μ in thickness and as many as thirty may be extracted from a single nucleus. They withstand maltreatment and can only with difficulty be crushed by pressure from a microneedle. They give protein reactions, but their infectivity has not been tested (Sheffield, 1941). Most of these crystals were too large to be usefully examined in the electron microscope but occasionally one was found sufficiently thin to be partially penetrated by the beam and to show some suggestion of internal structure (fig. 6).

(b) *Cytoplasmic Inclusions*.—These are similarly formed and have similar staining reactions to the amorphous bodies caused by the aucuba strain of tobacco mosaic virus. They sometimes contain a few birefringent particles of a size near the limits of resolution of the light microscope. In certain leaves, small doubly-refractive needle-shaped particles are formed within inclusions at a late stage. These are about 0.5μ wide and 2.5μ long. The bodies are less compact than those of aucuba mosaic but are more stable and consequently more readily handled by microdissection methods (Sheffield, 1941). To make preparations for examination in the electron microscope, a pipette with water held at the tip was plunged into the centre of the inclusion and then the pressure within the pipette was reduced so that some of the material composing it was drawn into the pipette. Some of the preparations were washed in water. Material was also taken out into $0.1\text{ M. (NH}_4)_2\text{SO}_4$ for comparison with the inclusions of tobacco mosaic virus.

Newly formed inclusions contained large quantities of material, mostly of a particle size below the limits of resolution of the electron microscope. A few small rhombic crystals were found. As the inclusion ages, the amorphous material decreases in quantity whilst the crystals increase in size and number. About 3 weeks after infection a few of the crystals may have sides of as much as 2μ but the majority are still below the limits of resolution of the light microscope. The larger crystals now visible are probably the birefringent particles occasionally seen between crossed Nicol prisms. These crystals are plate-like and of varying thickness. Some are quite impenetrable to the beam whilst others are so thin as to have very little stopping power to electrons. The crystals were embedded in a matrix of the amorphous material and sometimes in making the preparation some of them became shrunken and moved slightly from the position which they had occupied. Fig. 7 shows such a crystal of the same shape but rather smaller than a clearer area of collodion which it now

overlaps. The clear area of collodion is crossed by some fibrous material. Other crystals appeared hazy because they were surrounded by fibrous material. In untreated preparations these fibres were found only in association with the crystals. Preparations of mixed cytoplasm and sap were examined in the electron microscope daily from the time of inoculation until the inclusions could be seen in the optical microscope (about 1 week), and inclusions were then extracted for electron microscope examination at intervals of a few days until about 8 weeks after infection, but at no time could the fibres be found free either in preparations made in water or in $(\text{NH}_4)_2\text{SO}_4$ solution. Some preliminary attempts were made to treat the extracted material with trypsin. Immediately after placing the drop of extracted material on the membrane, a drop of trypsin solution in phosphate buffer was added. The grid was placed in a moist chamber on a warm plate for 2 hours after which the specimen was dried and washed. Material was taken from cells at various times after infection and was treated with trypsin at pH 6 or pH 8. Rods were found, but the data are insufficient to show whether these were more frequent in any one type of preparation.

About 4 weeks after infection almost all the amorphous material had disappeared and micrograms showed very little except crystals. Crystals of another form were now present. These were thin, 50–100 $\text{m}\mu$ wide and often several microns in length. They were usually found in clumps mixed with the rectangular crystals (fig. 8). Sometimes they showed a reticulate internal structure (fig. 9) but this is thought to be most probably an artefact due to electron bombardment.

In preparations made at a late stage from selected leaves, the needle-like crystals already seen in the light microscope were found but these were too opaque to be usefully studied in the electron microscope.

(c) *Other Cell Contents.*—Mixed cytoplasm and cell sap was taken from cells which contained no inclusions visible in the optical microscope and also from cells which contained inclusions. The micrograms were similar to those of inclusions from comparable plants but the concentration of crystals, etc., was very much less.

DISCUSSION.

The amorphous inclusions produced by the aucuba strain of tobacco mosaic virus were known to contain virus because when isolated, carefully washed, and inoculated to healthy plants they caused infection (Sheffield, 1939). This result has now been confirmed by the presence of rod-shaped particles in electron micrograms of suspensions of these inclusions (Beale 1937). Bawden and Sheffield (1939) suggested that the hexagonal crystals found in plants infected with several strains of tobacco mosaic virus also contained virus but for technical reasons this could not be confirmed by infectivity tests. Micrograms of suspensions of these crystals contain rods like those seen in purified preparations of the virus. The suspensions were probably contaminated with some cytoplasm but the number of rods present seems to be too great to be entirely due to this cause

and it is almost certain that the hexagonal crystals contain virus. Earlier work (Sheffield, 1939) has also suggested that although virus was present in the rest of the cell it was more concentrated in the inclusions. This too is confirmed by the finding of relatively fewer rods in micrograms from mixtures of cytoplasm and sap than in those from extracted inclusions.

All workers on tobacco mosaic virus agree that most of the virus in purified preparations is contained in rod-shaped particles. Agreement is general as to the approximate diameter of the particles and the fact that they are easily caused to aggregate end to end. All micrograms published show considerable variation in the length of the rods but some workers hold that the basic unit of tobacco mosaic virus is $280\text{ m}\mu$ long and explain the presence of the many rods which are shorter or not exact multiples of this figure as being artefacts. Gaw and Stanley (1947), whilst publishing no micrograms of controls, attempt to explain a particularly variable set of rods by claiming that treatment in a rocking dialyser was responsible for both aggregation and breaking. Bawden and Pirie (1945) suggest that the primary virus particle is small and that the amount of aggregation which occurs is determined by the treatment to which the virus is subjected in the purification processes. The micrograms of Crook and Sheffield (1946) confirm the presence of small particles in the preparations made by Bawden and Pirie and the fact that these particles aggregate when subjected to certain treatments.

In micrograms obtained from inclusion bodies, rods of many different lengths are seen and the range covered seems to be much the same as that found in purified preparations. The rods from hexagonal crystals seem to be on the whole shorter than those from the amorphous bodies suggesting the possibility that the virus in the former exists in smaller particles than in the latter. However, it is not claimed that this work offers any real evidence as to the shape of the virus particle when it is first formed or as it exists within the living cell. By shaving hairs from the leaf surface, crushing them on a glass slide and transferring the contents in water to a specimen grid, Oster and Stanley (1946) claim to have examined the virus as it exists within the living cell. It is impossible to extract any part of the cell contents without causing some injury and we do not know what effect such injury has on the forces within the cell nor on the state of aggregation of the virus particle. That the effect of injury in these cells is rapid and considerable is shown by the immediate breaking of hexagonal crystals into fibrous bodies if the wall of the cell containing them is momentarily pierced by a needle of 1μ diameter. Oster and Stanley also allowed the virus to dry in the presence of cell salts which alone would cause aggregation (Bawden and Pirie, 1946). Similarly the $(\text{NH}_4)_2\text{SO}_4$ used in the present studies may have caused the virus particles to aggregate. Oster and Stanley have argued "on the basis of probability considerations and from the diffusion constants of hypothetically smaller particles that no appreciable aggregation of such particles to form rods $280\text{ m}\mu$ in length could take place from the time the hair cells are broken until the final drying of the same." Their argument must be based on an assumption that the virus particles are evenly dispersed throughout the cell. That it is fallacious is shown by the way some of the amorphous inclusion bodies are formed (Sheffield, 1931), and by the virus content of these bodies and of

hexagonal crystals relative to that of the rest of the cell. Using their technique, Oster and Stanley might well have obtained the virus mainly from the hexagonal crystals, which would dissolve under such treatment, and where the virus particles are probably close together. Evidence on the state of aggregation of the virus in the living cell might be obtained by electron microscopic examination of infected cells grown in tissue cultures. It is doubtful whether this would be possible with plant cells for technical reasons. Even if it proves to be possible the effect of drying the virus in the presence of other cell constituents could not be ignored. The presence of a relatively high proportion of rods of about $280\text{ }\mu$ seen in some micrograms suggests that rods of this length may be more stable *in vitro* than those of other lengths but there is no evidence at all that a unit of such a size exists within the living cell.

This work lends some support to the suggestion that the particles of severe etch virus are rod-shaped as was deduced from indirect evidence by Bawden and Kassanis (1941). The only fibrous particles found in untreated material extracted from any parts of cells of plants infected with this virus was always in association with crystals. The fibrous material may be the virus but until some further evidence is found, it is impossible to be certain that it is not an artefact due to drying. However, the examination of partially purified preparations of the virus and the results of treating material extracted from infected cells with trypsin suggest the particles to be similar in form to those of tobacco mosaic virus.

Similarities and differences between the amorphous inclusions produced by the aucuba strain of tobacco mosaic virus and that induced by severe etch virus have already been discussed (Sheffield, 1941). The finding of crystals too small to be seen in the light microscope in the amorphous inclusions due to the latter virus shows a further parallel between the effects of these two unrelated viruses. Both inclusions were already known occasionally to give rise to needle-like fibres, those of aucuba mosaic regularly produce hexagonal crystals and it is now known that those of severe etch regularly give rise to crystals of a rhombic form. These are, however, far more stable than the hexagonal crystals of tobacco mosaic virus. The nature of the crystals formed both in the nucleus and in the cytoplasmic inclusion as a result of infection with severe etch is at present uncertain. The finding of similar nuclear inclusions in presumed healthy plants has already been discussed (Sheffield, 1941). As healthy cells sometimes contain crystals similar in appearance to those found in such great numbers in the inclusion bodies associated with severe etch virus, it may be that the virus causes the host to produce excessive quantities of some normal plant constituent. By analogy with tobacco mosaic virus, it is tempting to suggest that the crystals contain virus. Attempts to purify the virus seem to show that the virus content of plants reaches a peak about 2 weeks after infection and then falls off rapidly. If the virus were contained in the crystals in some not readily available form, it would be difficult to isolate. As the amount of virus which can be extracted from the plant drops, the crystals are increasing in size and number presumably at the expense of the amorphous material which simultaneously decreases. Considerably more work needs to be done on these crystals before any definite conclusions can be drawn.

SUMMARY.

Using micro-dissection methods, various parts of the contents of healthy and virus infected cells were isolated and prepared for examination in the electron microscope. Mixed cytoplasm and sap from healthy cells consisted chiefly of small particles varying in size from 20 μ to below the limits of resolution of the electron microscope. In the amorphous and crystalline inclusions due to strains of tobacco mosaic virus, rods of about 15 μ width and various lengths were found. Virus in smaller quantities was also found in mixed cytoplasm and sap from infected cells. In the amorphous inclusions due to severe etch virus, many crystals of various shapes too small to be seen in the light microscope were found. There is some evidence to support the suggestion that the particles of this virus are rod-shaped.

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DESCRIPTION OF PLATE.

All figures were taken with an RCA (type EMB2) electron microscope at an accelerating potential of 45–50 KV. Micrograms were enlarged photographically $\times 2$ and reduced by one-third in reproduction. The approximate magnification is given after each microgram.

- Fig. 1.—Mixed cytoplasm and sap from a healthy cell ($\times 20,000$ approx.).
 Fig. 2.—Material from an amorphous inclusion body produced by the aucuba strain of tobacco mosaic virus ($\times 20,000$ approx.).
 Fig. 3.—Material from a hexagonal crystal due to tobacco mosaic virus ($\times 20,000$ approx.).
 Fig. 4.—Partially purified preparation of severe etch virus ($\times 14,500$ approx.).
 Fig. 5.—Material from a cytoplasmic inclusion due to severe etch virus treated with trypsin at pH 6 ($\times 14,500$ approx.).
 Fig. 6.—Crystals from the nucleus of a plant infected with severe etch virus ($\times 7500$ approx.).
 Fig. 7.—Material taken from a cytoplasmic inclusion due to severe etch virus. The rhombic crystal has shrunk and moved during the making of the preparation leaving a cleaner area of collodion lined with fibrous particles ($\times 12,500$ approx.).
 Fig. 8.—Elongated crystals from cytoplasmic inclusion due to severe etch virus ($\times 12,500$ approx.).
 Fig. 9.—Crystals similar to those in fig. 8. The microgram is over-printed to show internal structure ($\times 14,500$ approx.).

ABSTRACTS AND REVIEWS

TECHNICAL MICROSCOPY.

Apertometry.—D. S. SPENCE ("Measurement of Numerical Aperture," *The Microscope*, 1947, 6, 253–9). Difficulties associated with the usual methods of measuring numerical apertures are discussed and a new type of apertometer is described. The microscope is set up with its tube horizontal and is focused on a fine needle hole mounted on the stage. Substage attachments and eyepiece are removed and the image of an index mark, placed some yards away, is viewed in or near the back focal plane of the objective. The rotation necessary to bring the image of the index from one side of the field to the other is measured with the stand mounted on a turning platform, moving round an axis on a fixed platform, with a pointer passing over a scale of degrees. A method of constructing a direct reading apertometer is also suggested. B. O. P.

Contrast in the Microscope Image.—A. F. HALLIMOND ("Production of Contrast in the Microscope Image by means of Opaque Diaphragms," *Nature*, 1947, 159, 851–2). The explanations of the phase-contrast effect given by Zernike and later by Kohler and Loos ignore the effect due to the inclination of the surfaces which has long been employed by mineralogists for refractive index determination in the Becke line and Schroeder tests. The name "stop contrast" is suggested for the method which is described. A narrow annular opening is placed below the condenser with a conjugate annular opening near the upper principal focus of the objective. Light deviated by the object is eliminated by surrounding the open annulus at the objective with an opaque diaphragm which does not in general cover the whole remaining aperture. The necessary width of the opaque portion of the diaphragm varies with the power and numerical aperture of the objective. Comparison photographs with different types of object are given. B. O. P.

Phase-contrast Microscopy.—L. C. MARTIN ("Phase-contrast Methods in Microscopy," *Nature*, 1947, 159, 827–30). The theoretical basis of the phase-contrast method is stated and various implications arising from the special conditions which apply are considered. Methods by which phase plates can be made are described and mention is made of a new phase-contrast microscope in which a controlled variable amplitude for the direct light component is obtained. The complete theoretical interpretation of the image is not yet fully understood and much work remains to be done, particularly with regard to the effects to be observed when thick objects are used. B. O. P.

Phase-contrast Microscopy.—E. W. TAYLOR ("The Control of Amplitude in Phase-contrast Microscopy," *Proc. Roy. Soc. A.*, 1947, 190, 422–6). An optical system is described whereby the amplitudes of the direct and diffracted beams forming the final image may be varied at will. Plane polarized incident light is used in conjunction with a condenser annulus of the usual form and a phase plate consisting of an inner annulus of left-handed quartz and a central disc and outer annulus of right-handed quartz (or *vice versa*) cut so that the planes of polarization of the direct and diffracted

light are rotated in opposite directions. The required retardation is obtained by means of a normal annular phase plate. The rotation of an analyser, mounted between the phase plate and the observer's eye, enables a graduated succession of positive and negative phase-contrast and bright and dark field effects to be obtained. The variations which take place in the image plane are illustrated by means of a series of photographs.

B. O. P.

HISTOLOGICAL AND CYTOLOGICAL TECHNIQUES.

Preparation of Mould Mounts.—V. B. D. SKERMAN ("Simple Technique for the Preparation of Mould Mounts," *Aus. J. Exp. Biol. Med. Sci.*, 1946, **24**, 1 pl.) A method is described for removing mould cultures from the substrate medium by which permanent micro-mounts can be obtained without derangement of the mycelium. A thin culture is desirable and a drop of a mixture of 75 parts ether and 25 parts alcohol is lowered on to the culture. This is allowed to evaporate slightly, and with a 4 mm. loop a drop of the following solution is placed within the moistened area: Ether, 75 ml.; ethyl alcohol, 25 ml.; celloidin, 10 g.

This film may be removed in 1–3 minutes with the embedded mycelium and any adhering agar can be removed therefrom.

The film may be mounted in lactophenol blue and placed later in clear lactophenol. Preparations are rendered permanent by ringing with shellac.

If it be necessary to remove the celloidin this should be done before it becomes too dry with a mixture of ether 75 parts and alcohol 25 parts, by this means dense cultures may be dissected out and stained as usual with lactophenol blue.

For photographic purposes the author recommends as follows: the film with the contained mycelium is immersed in dilute lactophenol blue for 10 minutes, washed in water, placed in warm (ca. 45° C.) alcohol until stain no longer runs freely from it, remove celloidin, tease out if necessary and mount in clear lactophenol. F. C. G.

Reclaiming Dried Zoological Specimens.—H. J. VAN CLEAVE and J. A. ROSS ("A Method for Reclaiming Dried Zoological Specimens," *Science*, 1947, **105**, 318). Small specimens after preservation in alcohol, are discarded as useless. Trisodium phosphate in dilute solution is very useful for reclaiming both large and small dried specimens: 0.25–0.5 p.c. of commercial trisodium phosphate in distilled water is satisfactory. Warmed solutions are more rapid in action than cold. For dried leeches 2 days at 35° C. was sufficient to restore normal appearance. Insects and worms can also be treated.

G. M. F.

Thin Sections for Electron Microscopy.—H. C. O'BRIEN, Jr., and J. W. BAIN ("Thin Undistorted Sections for Electron Microscopy," *J. Amer. Chem. Soc.*, **68**, 1139). To obtain thin sections of 0.1 μ thickness use must be made of a high speed ultramicrotome. Even sections of a soft plastic body such as soap can be obtained without distortion provided the sections are caught directly upon 200-mesh wire gauze without coming in contact with any surface.

G. M. F.

A Quadruple Stain for Animal Tissues.—L. J. GROVERMAN ("A Modified Quadruple Stain for Animal Tissues," *Trans. Kansas Acad. Sc.*, 1941, **44**, 414–5). Paraffin sections are brought down to 70 p.c. alcohol and stained in safranin O for 24 hours (0.5 g. safranin O, 50 ml. 50 p.c. ethyl alcohol solution, 50 ml. ethylene glycol monomethyl ether, 40 ml. formalin, 0.5 g. sodium acetate): rinse in tap water; stain with a 0.1 p.c. aqueous solution of gentian violet for 1½–2 minutes. Rinse in running water. Rinse for 30 seconds in equal parts of 95 p.c. ethyl alcohol and 1 part ethylene glycol monomethyl ether. Immerse in fast green F.C.F. solution for 60 minutes (a saturated solution in equal parts of clove oil and ethylene glycol monomethyl ether):

1 part is then added to 2 parts of 95 p.c. ethyl alcohol. Rinse for 30 seconds in a mixture of 2 parts 95 per cent ethyl alcohol and 1 part ethylene glycol monomethyl ether. Immerse in gold orange preparation for 45 minutes) prepared as for fast green F.C.F. solution). Rinse in a wash made up of equal parts of clove oil, absolute ethyl alcohol oxytol. Rinse in xylol and absolute ethyl alcohol, xylol and mount. Cartilage stains, bright red, nuclei purple, connective tissue green and muscle light red. In the intestines the submucosa takes on the gold orange, the goblet cells of the mucosa, the fast green, other mucosal cells gentian violet. G. M. F.

Sealing Liquid mounting Media on Microscopic Slides.—E. S. BARGHOORN ("The Use of Phenol Formaldehyde and Vinyl Resins in Sealing Liquid Mounting Media on Microscope Slides," *Science*, 1947, **106**, 299–300). Plant materials mounted in glycerin, lactic acid, and lactophenol are difficult to protect by sealing. The best phenolic resin for this purpose is a *p*-phenyl phenol formaldehyde compound containing tung oil and a metallic soap as oxidizing agent (Bakelite Corporation, New York City); its trade name is Tufon 74. A vinyl acetate resin dissolved in 95 p.c. ethyl alcohol, butyl acetate, dioxane, ethylene dichloride, and other organic solvents. G. M. F.

The Aceto-carmin Smear Technic.—L. SMITH ("The Aceto-carmin Smear Technic," *Stain Technol.*, 1947, **22**, No. 1, 17–31, 4 figs., 24 refs.). Full details are given of the aceto-carmin smear technique, beginning with the method of collecting specimens. The exact details to be followed for making preparations of plant chromosomes at prophase, of condensed chromosomes at meiosis and mitosis, and of coiled chromonemata are given in full. In addition a schedule is given for converting temporary slides into permanent mounts, and also for making smears of chromosomes in the salivary glands of *Drosophila*. The paper does not lend itself to abstraction and should be consulted by those interested. G. M. F.

A Method for the Serial Transfer of Minute Specimens.—J. B. CROSS and D. EBEN ("A Safe and Easy Method for the Serial Transfer of Minute Specimens through Various Reagents into Paraffin," *J. Parasitol.*, 1947, **33**, No. 1, 86). Minute specimens such as mites and tissue fragments are often difficult to detect when they have become translucent. Small pieces of lightweight bond paper, about 1 sq. mm., are marked with a pencil on one side and dipped rapidly into albumin fixative. The excess of fluid is drained off and the papers partially dried. Under the dissecting binocular microscope place a small drop of albumin on the unmarked side of one of the slips. By means of a needle deposit the object in the drop and quickly float the paper slip on the surface of the fixing fluid, marked side up. After fixation examine under the dissecting microscope and trim the slip so that the long axis is parallel with the long axis of the specimen. The slip with the specimen attached is now readily carried through dehydrating and clearing agents. In embedding fill a container with paraffin and lay the paper slip, marked side uppermost, on top of the hot paraffin. Cool quickly in ice water. Trim the paraffin block in accordance with the long axis of the object. Before cutting sections peel the paper away. G. M. F.

Acid Phosphatase in Germinating Maize Kernels.—O. E. KUGLER and E. H. BENNETT ("Histochemical Localization of Acid Phosphatase in Germinating Maize Kernels," *Stain Technol.*, 1947, **22**, 9–15, 7 figs.). Sections of germinating maize kernels are prepared by the freezing technique and studied by Gomori's method. For crude demonstration of acid phosphatase sections of 100–120 μ in thickness are used, for cytological study sections of 60–80 μ are cut. Moog's incubating mixture was used: acetate buffer at pH 4.7, 2.5 parts; 5 p.c. lead nitrate, 1 part; distilled water, 15 parts; 2 p.c. sodium glycerophosphate, 3 parts. After standing overnight

in a refrigerator the mixture is centrifuged to rid it of precipitated lead β -glycerophosphate, the clear supernatant being used as an incubating mixture. The incubated sections are rinsed in three changes of distilled water for 2 minutes and to remove lead-protein and lead glycerophosphate in 2.5 p.c. acetic acid for 4 minutes ; then in three changes of distilled water for 3 minutes. Sections are placed for 2 minutes in dilute ammonium sulphide solution, followed by several changes in distilled water and tap water ; they are then treated with a 1 : 1 mixture of xylene and distilled water and mounted in clarite. Sites of phosphatase activity stain brown or brownish-black.

G. M. F.

Rapid Staining of Frozen Sections.—W. V. COLE (" Quick Staining Method for Frozen Sections," *Stain Technol.*, 1947, **22**, 5-7, 2 figs.). A glass "spot plate" is required and a perforated section lifter of such a size as to fit one of the depressions in the spot plate. A bowl with a top diameter of 18 mm. and a 163-mm. handle soldered to one side of the bowl at right-angles is convenient. Sections are cut at 5-15 μ and placed in distilled water. The stains and reagents of the particular method to be used are placed in the spot plate in the order needed. For Mayer's alum hæmatoxylin 15 seconds immersion is long enough with 5 seconds in each of the successive alcoholic solutions. Fifteen seconds in the clearing solution are sufficient.

G. M. F.

The Microscopical Examination of Milk.—D. B. SHUTT (" A Rapid Staining Method for the Microscopical Examination of Milk : A Preliminary Note," *Stain Technol.* 1947, **22**, 1-2, 2 refs.). In the routine examination of milk with the Breed microscopical technique speed in the staining of the smear, coupled with a clear background are important factors. By the method here described the milk smear can be defatted, fixed, and stained in one operation in from 5 to 15 seconds, and the background can be decolourized. The formula is as follows :

Ether	...	...	...	...	...	...	100 ml.
Methyl alcohol, absolute	...	...	...	...	...	...	100 ml.
Methylene blue	...	...	...	...	...	...	1 g.
Hydrochloric acid	...	...	...	...	...	...	0.7 ml.

The ether and the methyl alcohol should first be mixed together before the methylene blue is added. As soon as the dye is in solution the hydrochloric acid can be included. The solution is held in a stoppered bottle and kept in the refrigerator when not in use. The milk smear is dried, dipped in the staining solution : 15 seconds will give a completely decolourized background ; 5-10 seconds a faintly blue background.

G. M. F.

Staining Malaria Parasites.—M. KOPEL (" A Modification of the Giemsa Stain for Malaria Parasites," *J. Tech. Methods*, 1945, **25**, 61-2). In place of absolute methyl alcohol in the Giemsa stain for malaria parasites, absolute ethyl alcohol may be used both for fixing the smear and in the stain itself. The cytoplasm of the parasites stains blue, the chromatin red.

G. M. F.

Tannin-iron III for Nucleoli and Plastids.—M. C. DE REZENDE-PINTO (" A New Cytological Technic, Tannin-iron III for Nucleoli and Plastids," *Stain Technol.*, 1947, **22**, 3-4, 2 refs.). Fix in either Carnoy (2 vol. absolute ethyl alcohol, 1 vol. glacial acetic acid), formol-saline ; or "acetoforsaldier" (immerse for 24 hours in 5 p.c. acetic acid, then for 48 hours in formol saline, rinse for 24 hours in running water, and finally immerse for 3-6 days in 3 p.c. aqueous potassium bichromate). Embed in paraffin. Hydrolyse. Bring sections down to water, rinse in cold normal hydrochloric acid ; hydrolyse in hot normal hydrochloric acid (58-60° C.) for 4 minutes for

material fixed in Carnoy's fluid and 2 minutes for material fixed in "acetoforsaldier" or formol saline. Stain. Transfer sections to Salazar's acetic tannin for 5 minutes. (Salazar's acetic tannin is prepared by dissolving 6 g. tannic acid in 10 ml. glacial acetic acid) and then add 30 ml. distilled water. Wash in distilled water. Stain in 4 p.c. iron alum solution for about 5 minutes; wash thoroughly in running water; dehydrate and mount in Canada balsam.

G. M. F.

A Method for Isolating a Single Trypanosome.—L. M. YUTUC ("The Hanging-drop Method of Isolating a Single Trypanosome and its Inoculation into White Mice," *J. Parasitol.*, 1947, 33, No. 1, 85). A drop of blood containing 5–10 trypanosomes per microscopic field under the low-power objective is placed on a plain slide. The drop is then diluted with three or more drops of sterile serum. The material is stirred till an even suspension is attained. A hollow-ground slide is placed under the microscope. With a sharp pointed instrument such as a tooth pick a tiny drop of fluid is removed from the diluted blood, placed on a cover-glass, inverted, and examined under the low power. If more than one trypanosome is visible the preparation is discarded.

G. M. F.

Chromosomes in Plants.—H. E. WARMKE ("Precooling combined with Chromosmo-acetic Fixation in Studies of Somatic Chromosomes in Plants," *Stain Technol.*, 1946, 21, 87–9, 1 fig., 3 refs.). The treatment of living excised roots in vials partially fitted with water at 0° C. for 90 minutes before fixation in cold Benda fluid at 0° C. shortens the chromosomes and preserves details of chromosome morphology in a number of plant species. Portions $\frac{1}{4}$ – $\frac{3}{4}$ inch in length are snipped off the exposed root tips with a fine forceps, washed in water with a camel's hair brush, and placed in ice cold water. The Benda formula used contains chromic acid 1 p.c., 16 ml., osmic acid 2 p.c., 4 ml., and glacial acetic acid 2 drops.

G. M. F.

Reconditioning Formaldehyde.—R. CARES ("A Note on Stored Formaldehyde and its Easy Reconditioning," *J. Tech. Methods*, 25, 67–70). Formaldehyde stock solutions may become cloudy from the formation by polymerization of para-formaldehyde or become acid because of the formation of formic acid by oxidation. When polymerization has occurred the milky solution should first be shaken to suspend the sediment; the fluid should then be poured into Mason jars and sealed tightly; the jars should then be autoclaved for 30 minutes at 15 lb. pressure. The water-clear solution may then be stored or diluted for use. Dilute 10 p.c. solutions become acid more readily than stock solutions of approximately 10 p.c. Marble chips may be used for neutralizing dilute solutions.

G. M. F.

Mounting Microscopic Arthropods with Soft Coverings in Lactic Acid.—H. GISEN ("Le montage à l'acide lactique d'arthropodes microscopiques à téguments mous," *Mitt. Schweiz entomolog. Gesellschaft*, 1947, 20, 581–6, 1 fig.). Insects are fixed either in 70 p.c. alcohol or preferably in the following mixture, alcohol (90–95 p.c.) 750 ml., ether 250 ml., acetic acid 30 ml., and formol (40 p.c.) 3 ml. for 2 days. If it is desired to examine the insects they are placed in lactic acid 100 g., acetic acid 4 g., gelatine 4 g.; when they are to be mounted permanently they are placed in lactic acid 100 g., gelatine 8 g.

G. M. F.

The Specificity of the Feulgen Reaction.—E. O. DODSON ("Some Evidence for the Specificity of the Feulgen Reaction," *Stain Technol.*, 1947, 21, 103–6, 5 refs.). The specificity of the Feulgen reaction for the detection of thymonucleic acid first

introduced by R. Feulgen and H. Rossenbeck (*Ztschr. physiol. Chem.*, 1924, **135**, 203) has been attacked by J. G. Carr (*Nature*, 1945, **156**, 143) on the grounds that it is a simple adsorption due to the fact that chromosomes ordinarily concentrate stains out of dilute solution. Biochemists ordinarily isolate nuclei from cytoplasm by hydrolysis with dilute acids. The selectivity of the Feulgen reagent for the nucleus would then depend on the destruction of the cytoplasm by acid hydrolysis. A chemical reaction between decolourized fuchsin and an aldehyde should be blocked by pre-treatment of the tissues with SO₂ water. Dodson takes up these points. Digesting tissue with nuclease to remove thymonucleic acid makes the chromosomes Feulgen negative though staining the digested chromosomes with ninhydrin reagent shows the protein skeleton still intact. The second point was tested by hydrolysing fixed tissues, washing off solutes, drying and comparing weight loss with controls. As differences were negligible the fixed cytoplasm must not have been made soluble by hydrolysis. The third point is chemically unsound. G. M. F.

CYTOLOGY.

The Structure of Cytoplasm.—LUDWIG MONNE ("Investigations into the Structure of the Cytoplasm," *Arkiv för Zoologi*, 1945, **36** A., No. 23, 1-28). The material studied consisted chiefly of the egg cells of *Psammechinus miliaris*, *Echinocardium cordatum*, and *Brissopsis lyrifera*. A Leitz biological polarization microscope provided with a Leitz rotating mica plate was used to analyse birefringence phenomena. The cells were treated with a variety of fixatives, including Bouin, Carnoy, Regaud, Champy, and Fleming. Sections were stained with various dyes including Unna's stain, toluidine blue, eosin, acid fuchsin, chlorophyll, and scarlet R. Numerous hæmatoxylin methods were found to give fine preparations; the sections were treated with a mordant (4 p.c. ferrous or ferric chloride in absolute alcohol or distilled water) for 6-12 hours, quickly rinsed, stained in 0.25 p.c. hæmatoxylin for 6-24 hours, and differentiated in aqueous 1-4 p.c. ferri-ammonium sulphate.

The ground cytoplasm of the egg cells studied is a texture rather than a network of fibrils which are visible by means of an ordinary light microscope. Chromidia containing ribosenucleic acid are embedded in the substance of the cytoplasmic fibrils which consist of bundles of polypeptide chains and are said to be nucleo-protein in nature. Nucleic acid occurs only at folded zones of the polypeptide chains where orientation is at a minimum. It is absent from regions where the chains lie parallel. Folded zones probably consist mainly of histones. The cytoplasmic fibrils are self-reproducing and new material is laid down between zones containing nucleic acid.

Chromidia are most easily distinguished in material fixed in fluids which dissolve lipoids. They contain varying amounts of nucleic acid which determines their staining reactions. A low concentration of nucleic acid is indicated by an affinity for acid dyes; high concentrations produce pronounced basophilia of the cytoplasm. Chromidia function as centres of protein synthesis but may form lipoid and possibly give rise to mitochondria and Golgi bodies.

Cytoplasmic fibrils may be transformed into specific cytoplasmic components such as myofibrils. The arrangement of fibrils may be irregular and can be modified by ultra-centrifugation and by hypertonic solutions. Microscopical inclusions such as mitochondria occur in the interstices of the meshed fibrils. In egg cells the fibrils are condensed beneath the cortex, at the surface of the nucleus, at one pole of the cell, and around the centrosome. Different cleavage patterns are due to asymmetrical arrangements of fibrils in the egg.

The staining reactions of living and fixed protoplasm were studied; the effects of lipoid-dissolving and preserving fixatives and of lipoid-soluble and insoluble dyes respectively were contrasted. The structural changes caused by hypertonic solutions are discussed. The condensation of the cytoplasmic fibrils results in concentric zoning

of the egg—cortex, yolk layer, mitochondrial layer, and ground cytoplasm layer containing the nucleus and vacuoles. Centrifugation of cells treated with hypertonic solutions for 1 hour at 10,000 times gravity resulted in the appearance of a "heavy drop" which does not develop in centrifuged normal eggs. A. K. P.

Lipines in Golgi Apparatus.—A. J. CAIN ("Demonstration of Lipine in the Golgi Apparatus in Gut Cells of *Glossiphonia*," *Quart. J. Micro. Sci.*, 1947, **88**, 151-7). The material used for this investigation was the Rhynchobdellid leech *Glossiphonia complanata*. The author differentiates between "lipoids" to include fats and all other substances that occur in plants and animals and resemble fats in solubility, and "lipines" in which he refers to lipoids that yield fatty acids, phosphoric acids or galactose and a basic nitrogen compound. Histochemical proof is presented for the presence of lipine in the Golgi region. Baker's acid hæmatein test is used for lipines and also a Sudan black technique as follows: (1) Fix, postchrome, wash, embed in gelatine, and cut frozen sections exactly as for acid hæmatein test; (2) leave for a few minutes in 50 and 70 p.c. alcohol; (3) transfer to a saturated solution of Sudan black in 70 p.c. alcohol for 7 minutes or longer (the exact time does not matter); (4) pass through three lots of 50 p.c. alcohol, 30 seconds each; (5) rinse in distilled water and mount in Farrant's medium, or counterstain first if desired. One counterstained preparation is useful if the tissue is not familiar.

When the alimentary canal is not distended the epithelial cells are columnar in shape and the main mass of the Golgi apparatus lies distal to the nucleus (i.e. on the side towards the lumen of the gut), it occasionally extends down the side of the nucleus or even surrounds it. By standard methods the Golgi material appears as uncoloured globules nearly always surrounded by a shell of osmiophil and argentophil substance. If these globules are above the lower range in size their variation is considerable in this respect, they are usually arranged in rows lying in the direction of the long axis of the cell. Nile blue after Lison is reported to give excellent differentiation in both stomach and intestine Golgi apparatus, it appears darker blue than the surrounding cytoplasm and slightly redder in tone. Globules of fat are coloured red so confusion is unlikely.

The positive result with Baker's pyridine extraction test shows that the principal lipid of the stomach Golgi is lipine. It is suggested that this material is composed of fat-free globules with lipid shells containing lipine. The apparatus in the intestine is different in one important point, that, where it is visible after acid hæmatein it is seen to contain little or no lipine by that test, the Sudan black technique shows that it contains about the same amount of lipide as the stomach. The amount of lipide in the rest of the cell varies inversely with that in the Golgi apparatus, little in the stomach and usually a great deal in the intestine. Mitochondrial concentration appears to be the same in both. It is suggested that the abundance of lipine in the intestinal cells is correlated with the active role of this organ in food absorption.

F. C. G.

FORAMINIFERA.

Foraminifera from Venezuela.—J. A. CUSHMAN ("A Foraminiferal Fauna from the Santa Anita formation of Venezuela," *Contrib. Cushman Lab. Foramin. Res.*, 1947, **23**, Pt. 1, No. 288, 1-18, 4 pls.). Two new species, one of *Gaudryina* and one of *Orbignyna*, are described from a rich fauna in the Santa Anita formation of Venezuela (many from the type section). This formation probably includes Late Cretaceous and Early Tertiary sediments. Relationship to other regions with the same specimens is noted.

G. H.

Foraminifera from Venezuela.—J. A. CUSHMAN (" *Lingulina naheolensis* Cushman, A New Name," *Contrib. Cush. Lab. Foram. Res.*, 1947, **23**, Pt. 1, No. 289, 18–19). The name *Lingulina naheolensis* is given to *Lingulina minuta* Cushman (not Franke) from the Naheola formation of the Paleocene. G. H.

Cuban Foraminifera.—J. A. CUSHMAN and P. J. BERMUDEZ ("Some Cuban Foraminifera of the genus *Rotalia*," *Contrib. Cush. Lab. Foram. Res.*, 1947, **23**, Pt. 2, No. 290, 23–9, pls. 5–10). Species of *Rotalia* from the Upper Cretaceous and Tertiary of Cuba are described. Many appear to be good index fossils with a possible wider distribution than is at present known. Ten new species are described. G. H.

Foraminifera from Florida.—J. A. CUSHMAN and E. R. APPLIN ("Two New Species of Lower Cretaceous Foraminifera from Florida," *Contrib. Cush. Lab. Foram. Res.*, 1947, **23**, Pt. 2, No. 291, 29–30, 1 pl.). The two new species are of *Trocholina* and *Cuneolina*. G. H.

Foraminifera from Trinidad.—J. A. CUSHMAN and H. H. RENZ ("Further Notes on the Cretaceous Foraminifera of Trinidad," *Contrib. Cush. Lab. Foram. Res.*, 1947, **23**, Pt. 2, 31–5, pls. 11, 12). The faunas of the Chadiere and Taroula formations underlying the Lizard Springs marl bear a relationship with the faunas of the Taylor and Santa Anita formations. A list of outcrop localities is given and the species are fully described including two new varieties. G. H.

Homonyms.—R. C. VAN BELLEN ("Some Homonyms in 'Foraminifera from the Middle Eocene in the Southern Part of the Netherlands Province of Limburg'," *Contrib. Cush. Lab. Foram. Res.*, 1946, **22**, Pt. 4, No. 286, 120–3). Homonyms in the original paper by van Bellen are rectified here. G. H.

Homonyms.—H. E. THALMANN ("Additional Homonyms in Foraminifera Erected since 1940," *Contrib. Cush. Lab. Foram. Res.*, 1946, **22**, Pt. 4, No. 287, 123–31). A comprehensive list of homonyms is given and the authors responsible for these are respectfully asked to re-name them. G. H.

Foraminifera from Ecuador.—J. A. CUSHMAN and R. M. STEINFORTH ("A New Species of *Amphistegina* from the Eocene of Ecuador," *Contrib. Cush. Lab. Foram. Res.*, 1946, **22**, Pt. 4, No. 283, 117–9, pl. 20). *Amphistegina elliotti* is described from beds assigned to the Middle Eocene. A list of associated fauna is given. G. H.

Foraminifera from Ecuador.—J. A. CUSHMAN ("The Genus *Ceratobulimina* and its Species," *Contrib. Cush. Lab. Foram. Res.*, 1946, **22**, Pt. 4, No. 282, 107–17, pls. 17, 18, 19). The species of *Ceratobulimina* Toulal 1920 and its subgenus *Ceratocancris* Finlay, 1939, are briefly reviewed, with notes on the stratigraphic distribution of each. G. H.

Foraminifera from Ecuador.—J. A. CUSHMAN and P. J. BERMUDEZ ("A New Genus *Cribropyrgo* and a New Species of *Rotalia*," *Contrib. Cush. Lab. Foram. Res.*, 1946, **22**, Pt. 4, No. 284, pp. 119–20, pls. 19, 20). The new genus *Cribropyrgo* is similar to *Pyrgo* but differing in aperture. It is a recent form from off Cuba. The new species of *Rotalia* is from the Gulf of Mexico. G. H.

Eocene Foraminifera.—W. S. COLE and P. J. BERMUDEZ ("Eocene Discocyclinidæ and Other Foraminifera from Cuba," *Bull. Amer. Pal.*, 1947, 31, No. 125, 1-36, 7 pls.). Five samples are analysed from Habana and Oriente Provinces, Cuba. The age is probably lower, middle, or higher, Lower Eocene, with reworked Upper Cretaceous. A new genus is described *Boreloides* of the family Alveolinellidæ with a new species for genotype. Three other new species are described and the genus *Eodictyoconus* Cole and Bermudez is suggested as referable to the family Cymbaloporidae. G. H.

Floridan Foraminifera.—W. S. COLE ("Internal Structure of Some Floridan Foraminifera," *Bull. Amer. Pal.*, 1947, 31, No. 126, 1-30, 5 pls.). Description from external appearance is not adequate for definition of some of the larger foraminifera, hence thin sections of some forms were made. The beccarii group of the genus *Rotalia* is placed in the genus *Turbinulina* Risso 1826. In all, seven forms are described and named on the basis of the internal structure. G. H.

Parisian Foraminifera.—Y. LE CALVEZ ("Revision des foraminifères lutétiens du Bassin de Paris : I. Miliolidæ," *Mem. Explicat. Carte Géol. de France*, 1947, 1-45, 4 pls.). A study is made of the Lutetian Foraminifera of the Paris Basin; also a revision of Terquem's collection. Some of Terquem's types are not considered to be valid. Incomplete and inexact definition have been emended in Terquem's collection. D'Orbigny's species are not figured, nor the larger Foraminifera (particularly Nummulites). The Miliolidæ and Rotalidæ constitute the major part of the Lutetian fauna, and it is with the Miliolidæ that this part of the study deals. Throughout the whole of the Eocene it is in the Lutetian that the Miliolidæ attain their peak. They are most abundant at Montmirail and Mécringes. Samples from twelve beds are studied and a systematic study is made of sixty-nine forms. Sixteen species are new and four are renamed. A table is given showing the frequency of the Miliolidæ in different beds. G. H.

Italian Foraminifera.—B. GRANELLO ("Sulla presenza dell' eocene medio fossilifero Presso Tulago (Trento)," *Riv. Ital. Paleont. Anno.*, 1947, 53, Fasc. I, 15-18). A fossiliferous Middle Eocene outcrop in the neighbourhood of Terlago lies conformably on Lower Eocene. This fold is covered by Lias as the result of a faulted fold. Six species of Nummulites are given in the text. G. H.

Foraminifera from the Hervian Plateau.—P. MOCAR, "Les sables à microfossiles du Plateau de Herve," *Bull. Geol. Soc. Belge.*, 1947, 70, Nos. 7, 8, 9, 10, 308-19). Sand with reworked Maastrichtian microfossils was recently discovered at the bottom of a solution cavity in the Hervian plateau. This sand presents very close analogy to the sands in the solution cavity of Battice, already described and where the same fossils, very rare, are found. From the proved geology of the region these sands were considered to be Oligocene, a conclusion reached after the study of heavy minerals. The descent by solution of these sands seems greater than their actual depth beneath the soil surface. The fauna listed is not sufficient to fix the definite age of the sand, but they do fix the age of the sand as younger than beds on the same level. G. H.

Foraminifera from British Somaliland.—C. D. OVEY ("A New Eocene Species of *Lockhartia* Davies from British Somaliland, with Notes on Other Species of the Genus, *Ann. Mag. Nat. Hist.*, August, 1947, B, No. 104, 571-6, 2 pls.). The species described is abundant in a whiteish limestone which may belong to the Allakkajid Beds of the Auradu Series (uppermost Lower Eocene). In addition all known species of *Lockhartia* (except *Lalveolata*) are figured for comparison. G. H.

PROTOZOA.

Nomenclature of *Pelomyxa*.—C. G. WILBER ("Concerning the Correct Name of the rhizopod, *Pelomyxa carolinensis*," *Trans. Amer. Micr. Soc.*, 1947, **66** (1), 99–101). A discussion on the correct generic name of *Pelomyxa carolinensis* which some authors include in the genus *Amœba*. The author believes that the multinucleate condition of the organism in question is a character of sufficient importance to justify its retention in the genus *Pelomyxa*. C. A. H.

Multiplication of Dysentery *Amœba*.—S. BRACKETT and A. BLIZNICK ("The Rate of Multiplication of *Endamœba histolytica* and its Relation to *in vitro* Drug Testing and Possibly to Nutritional Studies," *J. Parasitol.*, 1947, **33** (2), 154–66). Using a strain of *Entamœba histolytica* associated with a single species of bacteria, the authors studied its multiplication in culture, the numbers of amœbæ being counted with the help of a hæmocytometer. It was found that growth in culture could be obtained with an inoculum containing only 15 amœbæ, while 500 organisms invariably produced positive results. Moreover, the total output of amœbæ proved to be independent of the number introduced with the inoculum. The maximum fold increase in the population of amœbæ in a single transfer was 36,000 or about 15 generations. Under these conditions the average number of divisions per day was from 2 to 2½. Rapidly multiplying cultures were used to test the effect of drugs upon the amœbæ. C. A. H.

Excystation of Dysentery *Amœba*.—C. W. REES, L. V. REARDON, and F. E. JONES ("Observations on the Excystation of *Endamœba histolytica*," *J. Parasitol.*, 1947, **33**, 385).—In order to determine whether the cysts of *Entamœba histolytica* are able to hatch in the absence of bacteria isolated cysts were placed in microculture tubes through the wall of which they could be kept under microscopic observation. These tubes represent miniature test-tubes and are made from capillary glass tubing 1½ mm. in diameter. The cysts are introduced with a pipette into a micro-tube containing the requisite culture medium, after which the tube is sealed by flaming, to exclude contamination. In the microcultures without bacteria excystation occurred in all media except Locke's solution. Control cultures with a single species of bacteria showed only slightly higher rates of excystation than the bacteria-free cultures. C. A. H.

Culture of Dysentery *Amœba* on Solid Medium.—T. I. SNYDER and H. E. MELENKY ("Migration of *Entamœba histolytica* on Solid Media," *J. Parasitol.*, 1946, **32** (4), 354–8). Though *Entamœba histolytica* is usually cultivated on media comprising a solid slant, it requires a covering fluid for its development, whereas free-living amœbæ can be grown on solid surfaces without a covering fluid. This difference is thought to be due to anaerobic conditions produced in the fluid component by the action of the accompanying bacteria. The authors have developed a method of cultivation which enables the amœbæ in question to migrate away from the bacteria and establish themselves on a solid medium. The medium used for this purpose has the following composition: whole horse serum 25.0 p.c.; Difco yeast-autolysate 2.5 p.c.; Difco proteose peptone 0.5 p.c.; Bacto agar 1.5 p.c.; Ringer's solution 70.5 p.c.; cholesterol 1 µg. per ml. Its reaction is adjusted to pH 6.8–7.2. After autoclaving, the medium is dispensed in culture tubes, 4 ml. per tube, and degassed at a pressure of 10–20 mm. of mercury. The slanted media are then stored overnight *in vacuo*, finally any fluid accumulated at the bottom of the tubes is removed with a pipette, and the surface of the medium is dusted with rice starch. The slants are then inoculated by introducing at the butt one drop of stock culture of *E. histolytica* and the cultures are incubated at 37° C. Anaerobic conditions are provided either with the help of a vacuum pump or by absorbing oxygen with a mixture of pyrogallol and sodium carbonate. Under these conditions the amœbæ migrate on the solid slant beyond visible bacterial

growth and within a few days extend over the whole slant. Marked reduction of oxygen tension and increase of carbon dioxide tension appear to be essential for growth of *E. histolytica* on a solid medium. Subinoculations from slant to slant were successful but separation of amoebæ from bacteria was not achieved. C. A. H.

Structure of Flagella.—H. M. OWEN ("Flagellar Structure: I. A Discussion of Fixation and Staining of the Protozoan Flagellum," *Trans. Amer. Micr. Soc.*, 1947, **66** (1), 50–8, 1 pl.). The flagella of the Mastigophora are notoriously difficult objects to stain properly, with the result that there is considerable controversy regarding their intimate structure. At present two or three types of flagella are recognized: (1) The lash-flagellum which has a hair-like appendage on the distal end of the basal part, (2) the simple flagellum which is without appendages, and (3) the ciliary or flimmer-flagellum, provided with lateral pinnate appendages, which some authorities regard as artefacts. The author has made an evaluation of some of the existing techniques for showing the flagellar structure and has devised a standard method applicable to the flagella of various Mastigophora, with special reference to the flimmer-flagellum. For fixation he uses a solution of 2 p.c. osmic acid (10 parts) and 20 p.c. formalin (1 part). One or two drops of this solution is placed on the drop containing the organisms and the slide is allowed to dry at room temperature. Staining is carried out either by Loeffler's or Couch's methods. This study has shown that the flimmer-flagellum is only produced by dry-fixation but is not revealed after wet fixation. The lateral appendages are, therefore, regarded as artefacts of fixation due either to extrusion of the plasma through the flagellar membrane or to separation of the micelles of which the sheath is composed. The osmic-formalin fixative has been found to be the most reliable in bringing out the structural details of the flagellum, while Loeffler's staining method is the best for over-all staining. Couch's staining technique produces the best results in demonstrating the lash-flagellum. C. A. H.

New Euglena.—C. E. PACKARD ("Notes on the Euglenæ with Presentation of a Probable New Species," *Trans. Amer. Micr. Soc.*, 1947, **66** (1), 85–95, 5 text-figs.). Description of an unusual form of *Euglena* found in two ponds in U.S.A. The flagellate was attenuated with a stiff body truncated anteriorly and tapering off posteriorly, measuring 146.5–214.2 μ in length and 6.6–10.7 μ in breadth. Within the cytoplasm there were 12–14 green chloroplasts and a red eye-spot. A single contractile vacuole was situated near a flask-shaped cytopharynx which was 16.6 μ long. A flagellum could not be made out. Having failed to identify this flagellate with any euglenoids described in the standard works, which are discussed by the author, he proposes to regard it provisionally as a new species, under the name *Euglena crescentia* sp. n. C. A. H.

Immunity in Rat Trypanosomiasis.—D. L. AUGUSTINE ("Some Factors in the Defence Mechanism against Reinfection with *Trypanosoma lewisi*," *Proc. Amer. Acad. Arts. Sci.*, 1943, **75** (3), 85–93, 1 pl.). It has previously been shown that in the course of immunity acquired by rats against infection with *Trypanosoma lewisi* two anti-bodies are produced one of which (ablastin) inhibits reproduction of the parasite while the other is trypanocidal, causing the disappearance of the trypanosomes from the blood and leaving the rat immune to reinfection. The author has studied the defence mechanism to reinfection. It was formerly thought that trypanosomes inoculated intraperitoneal into immune rats were prevented from entering the blood stream by the lymph nodes, but the author has shown that they were incapable of preventing the trypanosomes from penetrating into the circulation. However, they remain in the blood only for a few days, after which they are immobilized and agglutinated by anti-bodies and finally destroyed. The author believes that a single

trypanocidal anti-body is involved in the defence mechanism against reinfection, and that the phenomenon is distinct from that in initial infections. C. A. H.

Oxymonad Flagellates.—J. B. CROSS ("The Flagellate Subfamily Oxymonadinæ," *Univ. California Pub. Zool.*, 1946, **53** (3), 67–162, 14 pls., 1 text-fig.). The author gives a systematic description of the subfamily Oxymonadinæ, which comprises flagellates parasitic in the intestine of termites. Among these the following five are new species. *Barcella coronaria*, *Oxymonas caudata*, *O. rotunda*, *O. megakaryosoma*, *O. notabilis*. A detailed account is given of the cytology and reproduction of a number of flagellates, with special reference to the nuclear processes. C. A. H.

Development of Gigantomonas.—H. KIRBY ("Gigantomonas herculea Dogiel: A Polymastigote Flagellate with Flagellated and Amœboid Phase of Development," *Univ. California Pub. Zool.*, 1946, **53** (4), 163–226, 7 pls., 13 text-figs.). This is a revision of the Polymastigote flagellate, *Gigantomonas herculea*, parasitic in the gut of the termite, *Microhodotermes viator*, in South Africa. The author has now established a new subfamily Gigantomonadinæ to include this genus. It is shown that *G. herculea* has a flagellate and amœboid stages. The former has three anterior flagella, a trailing flagellum, an axostyle, and a cresta, and resembles the Devescovinid flagellates, except for the absence of a parabasal body. The amœboid forms are considerably larger than the flagellate forms and represent the reproductive stage. By division, resembling that of Trichomonad flagellates, they give rise to flagellate forms. In the amœboid form nuclear division is sometimes arrested with the result that they remain connected by a centrodemose, thus resembling the aberrant amœba, *Dientamœba fragilis*, which is parasitic in man. In the flagellate in question the author found nine kinds of symbiotic micro-organisms. The paper is illustrated by a series of excellent plates. C. A. H.

Affinities of Trichomonads.—H. KIRBY ("Flagellate and Host Relationships of Trichomonad Flagellates," *J. Parasitol.*, 1947, **33** (3), 214–28). The author discusses the affinities of the Trichomonad flagellates and gives a revised classification group, which is designated as the order Trichomonadida. This order is characterized by the possession of organellæ arranged in mastigont systems, each comprising 3–6 flagella (one of which is a trailer), an axostyle and a parabasal body, all of which are associated with a nucleus. The Trichomonadida contain four families: Monocercomonadidæ (fam. n.), Devescovinidæ (with subfamilies Devescovininæ, Gigantomonadinæ), Calonymphidæ and Trichomonadidæ. The last-named family possesses an undulating membrane and costa, and comprises the genera *Trichomonas* (type *T. vaginalis*), *Tritrichomonas* (type *T. augusta*), *Pentatrichomonas* (type *P. ardinellei* with *Trichomonas hominis* as a synonym?), *Pentatrichomonodies* (type *P. scroa*), and *Pseudotrypanosoma* (type *P. giganteum*). C. A. H.

Trichomonad of Birds.—R. M. STABLER ("Trichomonas gallinæ, Pathogenic Trichomonad of Birds," *J. Parasit.*, 1947, **33** (3), 207–13, 1 text-fig.). An account of the structure bionomics and host-parasite relations of *Trichomonas gallinæ* parasitic in pigeons and some other birds. Morphologically it closely resembles the genital trichomonad of man (*T. vaginalis*) but lives in the upper regions of the alimentary canal of birds, whence it may secondarily invade the body cavity and produce damage to other organs, commonly causing the death of the host. It rarely penetrates the intestine below the gizzard and differs from the trichomonads found in the lower regions. *T. gallinæ* has been described under other names (*T. hepatica*, *T. columbae*, *T. diversa*, *T. halli*) which are now regarded as its synonyms. Transmission of the infection is affected through contaminated drinking water. C. A. H.

Trichomonad of Cattle.—B. B. MORGAN ("A Summary of Research on *Trichomonas fetus*," *J. Parasit.*, 1947, **33** (3), 201-6). A review is given of the present position of our knowledge regarding *Trichomonas fetus*, the causative organism of a venereal disease in cattle. This trichomoniasis has a world-wide distribution. In cows the parasite invades the vagina and uterus, causing symptoms varying in severity and including abortion, while in the bull the infection is found in the preputial cavity. Transmission from bovine to bovine occurs during the sexual act. The disease is diagnosed by finding the trichomonads in the genital exudates, by the culture method and by serological methods (complement fixation, agglutination, and intradermal test).

C. A. H.

Human Trichomonads.—D. H. WENRICH ("The Species of *Trichomonas* in Man," *J. Parasit.*, 1947, **33** (3), 177-83, 3 text-figs.). In this paper a review is given of the morphology, systematics, and host-parasitic relationships of the species of *Trichomonas* parasitic in man, one of which, *T. hominis* inhabits the intestine; another, *T. tenax*, the mouth; and the third *T. vaginalis*, the genital tract. Morphologically these three species are very much alike: the oral and genital trichomonads have four anterior flagella; an undulating membrane with a marginal flagellum, both of which are shorter than the body; a slender costa and a parabasal body. They differ in the larger size of *T. vaginalis*, shape of the nucleus and chromosome member. The intestinal parasite typically has five anterior flagella, an undulating membrane extending to the end of the body, with a marginal flagellum possessing a free trailing portion, and a stout costa. In the temperate zone the incidence of infection with *T. vaginalis* and *T. tenax* is much higher than that of *T. hominis*. These flagellates also differ in cultural requirements, *T. vaginalis* being unable to grow at room temperature, while the other two are able. Apart from morphological differences the independent nature of the three trichomonads is supported by the fact that they are not transferable to habitats other than those in which they are normally found.

C. A. H.

Physiology of Vaginal Trichomonad.—J. J. JOHNSON ("The Physiology of Bacteria-free *Trichomonas vaginalis*," *J. Parasit.*, 1947, **33** (3), 189-98). The author reviews the results of 7 years' work on the cultivation of *Trichomonas vaginalis* with the object of determining its nutritional requirements and the growth factors involved. Following the isolation of the flagellate from the accompanying bacterial flora, the author and his collaborators devised an improved medium approximating a chemically defined fluid, in which the effect of separate constituents could be determined. It was found that blood serum and liver infusion contain factors essential to the growth of the trichomonad, while peptone and maltose supply the protein and carbohydrate requirements. As regards reaction of the medium, maximum growth was obtained at pH 5.5-6.0. Since *T. vaginalis* behaved as a facultative anaerobe the medium was rendered as far as possible oxygen-free. Studies of temperature requirements showed that maximum multiplication occurs between 35° and 39° C., while it stops above 44° and below 25.5°. The addition of vitamins to cultures also brings about an increase in the population. Among the serum contents an ether-soluble fraction together with an ether-insoluble fraction were found to contain essential growth factors.

C. A. H.

New Myxosporidia.—P. A. MEGLITSCH ("Studies on Myxosporidia of the Beaufort Region: I. Observations on *Chloromyxum renalis*, sp.n., and *Chloromyxum granulorum* Davis," *J. Parasit.*, 1947, **33** (3), 265-70, 4 text-figs.; "II. Observations on *Kudoa chupei* (Hahn), gen. nov.," *ibid.* 271-7, 16 text-figs.). A description of new Myxosporidia from North American fishes. The first paper deals with a new species *Chloromyxum renalis* sp.n. parasitic in the collecting and mesonephric ducts of *Fundulus majalis*, and with *C. granulorum* recorded from a new host, *Bairdiella chrysura*, in which the urinary bladder is invaded. The structure and development of

these parasites is described and an account is given of the histopathological changes caused by the infections. There is a check list of species of *Chloromyxum* recorded since 1933. In the second paper a new genus *Kudoa* is created for *Chloromyxum clupei* (type). It is represented by histozoic Myxosporidia of the fam. Chloromyxidae, typically parasitic in skeletal muscle and characterized by spores of quadrate or stellate shape. The spore membrane is thin, with indistinct sutures; there are four polar capsules. The following species, formerly regarded as *Chloromyxum*, have been removed to the new genus: *Kudoa clupei* (Hahn, 1917); *K. quadratum* (Thélohan, 1895); *K. sp.* (Tyzzer, 1900) *p.p.*; *K. funduli* (Hahn, 1915); *K. sp.* (Davis, 1924); *K. thyrsites* (Gilchrist, 1924); *K. histolytica* (Pérard, 1928); *K. rosenbuschi* (Gelormini, 1944). C. A. H.

Development of Bovine Coccidium.—D. M. HAMMOND, G. W. BOWMAN, L. R. DAVIS, and B. T. SIMMS ("The Endogenous Phase of the Life-cycle of *Eimeria bovis*," *J. Parasit.*, 1946, **32** (4), 409–427, 3 pls., 3 text-figs.). An account is given of the endogenous life-cycle of *Eimeria bovis* in calves experimentally inoculated with ripe oocysts and killed at various intervals. Examination of the intestinal contents and faeces showed that excystation of sporozoites occurred in the small bowel 15–46 hours after inoculation. Early schizonts arising from sporozoites were found in the posterior half of the small intestine after 5 days, occupying cells in the lumen of the lacteals or in their walls. In the course of segmentation of schizonts merozoites are arranged radially. As they grow the schizonts assume macroscopic dimensions (281–203 μ) this being the stage previously referred to *Globidium* or *Gastrocystis*. Mature schizonts are first seen 14 days after inoculation, reaching maximum numbers after 16–18 days. There appears to be only one generation of schizonts. The sexual stages of this coccidium are produced in the caecum and colon about 17 days after inoculation. Discharge of oocysts takes place after 18–20 days. The pathological effect of the infection is associated with the sexual stages. C. A. H.

Avian Haemoprotozoa.—J. LAWRENCE ("Some Observations on the Plasmodia and Other Blood Parasites of Sparrows," *Proc. Linn. Soc. N.S. Wales*, 1946, **71** (1–2), 1–5). The author reports the results of examination of Australian sparrows for the presence of *Plasmodium* and other blood protozoa. Experimental infections of mosquitoes were also carried out, with the view to determine local vectors of avian malaria. Of ninety-one birds examined sixty-five (71 p.c.) were infected with malaria parasites, while twenty-seven harboured *Toxoplasma*. The author failed to find any morphological differences between *P. relictum* and *P. cathemerium*. *Culex fatigans* proved to be an efficient invertebrate host for the malaria parasites. C. A. H.

Haemoprotozoa of Bird.—M. J. TAKOS ("Blood Parasites of Some Panamanian Birds," *J. Parasit.*, 1947, **33** (3), 229–30). A report on the blood parasites of birds caught in Panama. Of a total of 40 birds, representing 23 species, 13 were infected with a *Haemoproteus*, a *Plasmodium*, unidentified Haemosporidia, microfilariae or a mixture of these parasites. The findings are shown in tabular form. C. A. H.

Malaria in Grouse.—A. M. FALLIS ("*Plasmodium circumflexum* (Kikuth) in Ruffed Grouse in Ontario," *J. Parasit.*, 1946, **32** (4), 345–353). A description of the morphology, life-cycle, and host-parasite relations of *Plasmodium circumflexum*, parasitic in the ruffed grouse of Canada. The parasite is elongated but does not always surround the host-cell nucleus in the grouse, though it does so in the case of the smaller erythrocytes of the canary. The average number of merozoites produced by schizogony is sixteen (maximum twenty-two, minimum ten), whereas in the canary the average number is nineteen. A comparison of the morphological characters of the grouse strain shows that its characters are midway between *P. lophurae* and *P. circumflexum*. The periodicity of development of the grouse *Plasmodium* is not clear-cut, varying

from 36 hours in some birds and 48 hours in others. The grouse parasite is slightly infective to turkeys, more so to ducks, and most to canaries, but is not pathogenic to its own host or other birds. C. A. H.

Protozoa Parasitic in Crane-Fly Larvæ.—F. W. LUDWIG ("Studies on the Protozoan Fauna of the Larvæ of the Crane-fly, *Tipula abdominalis* : I. Flagellates, Amœbæ, and Gregarines," *Trans. Amer. Micro. Soc.*, 1946, **65** (3), 189-214, 7 pls.). Description of various parasitic protozoa found in the larvæ of the crane-fly, *Tipula abdominalis*, collected in U.S.A. This is preceded by a list of such parasites reported between 1892 and 1932. Attached to the epithelium of the midgut were flagellates of the genus *Leptomonas*. The intestinal fauna also included *Rhizomastix gracilis*, *Retortamonas agilis*, *R. alexei*, *Monocercomonas trichoptera* var. *tipula*, *Tetratrichomastix parisii*, *Hexamastix tipula* (comb. nov. for *H. batrachorum* var. *tipula*), *Monocercomonoides melolonthæ*, *M. orthopterorum*, *Polymastix melolonthæ*, *P. wenrichi*, *Entamæba minichini*, *Endolimax* sp., *Actinocephalus tipula*. In the hæmocoel occurred a new gregarine, *Didymophyes electæ* sp.n. The generic name *Retortamonas* is used in place of *Embadomonas*, while *Monocercomonas* is employed for *Eutrichomastix*, and *Monocercomonoides* is substituted for *Monocercomonas*. The nomenclature of *Retortamonas*, *Monocercomonas*, and *Monocercomonoides* is discussed in detail.

C. A. H.

HELMINTHS.

A New Species of Cestode—*Choanotænia numenii* from the Long-billed Curlew.—R. L. OWEN (*Trans. Amer. Micro. Soc.*, 1946, **65**, 346-54, 4 figs., 1 table). Tapeworms collected from the intestine of the Long-billed Curlew—*Numenius americanus americanus* are described. Numerous specimens were available for examination and an exhaustive description is provided, placing the type in the genus *Choanotænia*. The history and taxonomics of the genera *Choanotænia* (Railliet, 1896) and *Paricterotænia* (Fuhrmann, 1932) are discussed at some length and a description of four closely related species of *Choanotænia* is given as a basis for comparison with the specimens under consideration. The differences in structure are too significant to include it among any of these species and a new species—*C. numenii*—has been created. The most nearly similar species *C. stercoraria*, differs in many important structural features from *C. numenii* and occurs as a parasite of *Stercorarius pomarinus* in Arctic Russia whereas the type described was collected in Nebraska.

The paper includes a small table showing the chief points of difference between the five closely related species of *Choanotænia*. O. D. S.

A New Nematode, *Procamallanus pereirai*, with a Key to the Genus.—R. F. ANNEXEAUX (*Trans. Amer. Micro. Soc.* 1946, **65**, 299-304, 3 figs.). The author describes male and female specimens of nematodes of the genus *Procamallanus*. Four specimens, one male, two mature females, and one immature female were found in the intestine of the "Jack Smelt"—"*Atherinopsis californiensis*"—collected at Bolinas Bay, California. Hitherto, no members of the genus *Procamallanus* have been reported from North America. A new species *P. pereirai* is created on the basis of difference in the number and arrangement of caudal papillæ, number of spirals present in the buccal capsule and some other unmentioned minor differences from its closely related species *P. amarali* (Vaz and Pereira, 1934). The female specimens bore superficial resemblance to two other species of the same genus but are separated on grounds of difference in structural measurements and position of the vulva.

A discussion of the genus *Thelazo* is included and on account of its similarity to the genus *Procamallanus* it is removed from the family Thelaziidae. The genus *Thelazo*, is however, still considered valid.

A key to species of the genus *Procamallanus* is included.

O. D. S.

PROCEEDINGS OF THE SOCIETY.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, OCTOBER 17TH, 1945, AT 5.30 P.M., DR. JAMES A. MURRAY, F.R.S., PAST-PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were taken as read and signed by the Chairman.

New Fellows.—The following candidates were balloted for and duly elected Ordinary Fellows of the Society :—

N. D. Constantine.	Hereford.
Noel Goodwill.	Malton.
O. Hildebrand.	London.
J. M. Watson, A.R.C.S.	London.
J. W. Waller Tabb.	Cheam.
Robert C. Enever.	Stevenage.
C. R. Russell, M.Sc.	New Zealand.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner :—

Rev. J. J. Moran.	London.
Kenneth Kirkby.	London.

Deaths.—The Chairman announced the regrettable loss to the Society, by death, of the following Fellows :—

C. D. Todd.	Elected 1938.
Evan MacLeod,	„ 1925.

A vote of condolence with the relatives was passed.

Donations were reported from :—

Dr. L. P. Clarke, F.R.M.S.—

A portable microscope of the Moginie type, by Swift, with accessories in case.

Executors of the late Mr. C. D. Soar—

23 boxes of micro-slides, *Hydracharina*, etc.,
4 boxes of lantern slides,
micro oil lamp and sundries,
collection of original drawings, note-books and miscellaneous reprints,
mainly on the *Hydracharina*.

Clay-Adams Co., Inc.—

"The Microscope and its Use," by F. J. Munoz and H. A. Charipper.
Votes of thanks were accorded to the donors.

Papers.—The following communications were read and discussed :—

Dr. F. W. Jane—

"*Clororhabdion*, a New Genus of the Xanthophyceæ."

Dr. R. J. Ludford, F.R.M.S.—

"Mitotic Poisons."

Votes of thanks were accorded to Dr. Jane and Dr. Ludford for their communications.

The following PAPERS were read in title :—

Walter J. Parr, F.R.M.S.—

"*Torresina*, a New Genus of the Foraminifera from Eastern Australia."

C. R. Russell, M.Sc., F.R.M.S.—

"A New Rotifer from New Zealand."

Announcements.—The Secretary made the following announcement :—

The next meeting of the Biological Section will be held on Wednesday, November 7th, 1945.

The Secretary also referred to the regrettable delay in publication of the 1944 issue of the Society's Journal which was due to enemy action. Printing was now again in hand and it was hoped the Journal would be available for issue shortly.

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, NOVEMBER 21ST, 1945, AT 5.30 P.M., DR. T. E. WALLIS IN THE CHAIR.

The Minutes of the preceding meeting were taken as read and signed by the Chairman.

New Fellows.—The following candidates were balloted for and duly elected Ordinary Fellows of the Society :—

Rev. J. J. Moran.
Kenneth Kirkby.

London.
London.

Paper.—The following communication was read and discussed :—

J. M. Watson, A.R.C.S., F.Z.S., F.R.M.S.—

“ Studies on the Morphology and Bionomics of a Little-known Holotrichous Ciliate—*Balantiophorus minutus* Schew.”

A vote of thanks was accorded to Mr. Watson for his communication.

Announcement.—The Chairman made the following announcement :—

The next meeting of the Biological Section will be held on Wednesday, December 5th, 1945.

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, DECEMBER 19TH, 1945, AT 5.30 P.M., DR. R. J. LUDFORD IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed and signed by the Chairman.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner :—

A. E. McClure.
Harold A. Peace.
L. W. Barsdorf.
Arthur D. Lloyd.
Owen Lawrence.
Cecil H. Kenyon.
Bernard L. Luscombe.

London.
Tring.
London.
Beckenham.
New Zealand.
Weston-super-Mare.
Hayling.

Donations were reported from :—

Mr. J. E. Barnard, F.R.S., P.R.M.S.—

“Interferenz-Erscheinungen in polarisirten light,” by Dr. Hans Hauswaldt. 1902, 1904, 1908.

“Foraminifera of the Kerimba Archipelago,” by E. Heron-Allen and A. Earland.

Miscellaneous parts of the Proceedings of the Physical Society.

Physical Society’s Report on the Relativity Theory of Gravitation by A. S. Eddington.

Physical Society’s Report on Radiation and the Quantum Theory by J. H. Jeans.

Transactions of the Optical Society. Vols. XVIII–XXXIII. 1917–1932.

Micrographic Dictionary. Vols. I and II. By Griffith and Henfrey.

A sheet of transparent silvered glass $12\frac{1}{2}$ in. $\times$ 9 in., 2·6/3·2 mm., by Pilkington Bros.

The British Museum (Natural History)—

“Acari as Agents transmitting Typhus in India, Australasia and the Far East,” by Susan Finnegan, B.Sc., Ph.D.

Prof. E. J. B. Verleyen, F.R.M.S.—

“Levensschets van Dr. H. van Heurch en overzicht van zijn verzamelingen,” by Prof. Verleyen.

Votes of thanks were accorded to the donors.

Paper.—The following communication was read and discussed :—

Mr. F. C. Grigg, F.R.M.S.—

“Recent Advances in Chromosome Smear Technique.”

A vote of thanks was accorded to Mr. Grigg for his communication.

Announcement.—The Secretary made the following announcement :—

The next meeting of the Biological Section will be held on Wednesday, January 2nd, 1946.

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, JANUARY 16TH, 1946, AT 5.30 P.M., DR. JAMES A. MURRAY, F.R.S., PAST-PRESIDENT, IN THE CHAIR.

The Minutes of the preceding meeting were read, confirmed, and signed by the Chairman.

New Fellows.—The following candidates were balloted for and duly elected Ordinary Fellows of the Society :—

A. E. McClure.	Mill Hill.
A. D. Lloyd.	Beckenham.
H. A. Peace.	Tring.
L. W. Barsdorf.	London.
Owen Lawrence.	New Zealand.
C. H. Kenyon.	Weston-super-Mare.
B. L. Luscombe.	Hayling.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner :—

P. S. Moss	Queensland.
M. J. Curran, B.Sc.	Hemel Hempstead.
J. K. Letts, F.Z.S.	Greenford.
C. C. Swatman.	Walthamstow.

Donation was reported from :—

John Wiley & Sons, Inc.—

“Electron Optics and the Electron Microscope,” by V. K. Zworykin, G. A. Morton, E. G. Ramberg, J. Hillier, and A. W. Vance.

A vote of thanks was accorded to the donors.

Paper.—The following communication was read and discussed :—

Mr. A. E. McClure, F.R.M.S.—

“The Superficial Illumination of Mounted Microscopic Objects by means of the Vertical Illuminator.”

A vote of thanks was accorded to Mr. McClure for his communication.

Announcement.—The Chairman made the following announcement :—

The next meeting of the Biological Section will be held on Wednesday, February 6th, 1946.

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, FEBRUARY 20TH, 1946, AT 5.30 P.M., MR. J. E. BARNARD, F.R.S., PRESIDENT, IN THE CHAIR.

The Minutes of the preceding meeting were read, confirmed, and signed by the President.

New Fellows.—The following candidates were balloted for and duly elected Ordinary Fellows of the Society :—

P. S. Moss.	Queensland.
M. J. Curran, B.Sc.	Hemel Hempstead.
J. K. Letts, F.Z.S.	Greenford.
C. C. Swatman.	Walthamstow.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner :—

Walter G. Hartley, B.Sc.	Helensburgh.
Arthur T. Campbell.	Brighton.

Paper.—The following communication was read and discussed :—

Mr. J. Smiles, A.R.C.S., F.R.M.S.—

“ Electron Microscopy in relation to Other Known Methods.”

A vote of thanks was accorded to Mr. Smiles for his communication.

Announcement.—The Secretary made the following announcement :—

The next meeting of the Biological Section will be held on Wednesday, March 6th, 1946.

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, B.M.A. HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, MARCH 20TH, 1946, AT 5.30 P.M., DR. JAMES A. MURRAY, F.R.S., PAST-PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed, and signed by the Chairman.

New Fellows.—The following candidates were balloted for and duly elected Ordinary Fellows of the Society :—

W. G. Hartley, B.Sc.	Helensburgh.
A. T. Campbell.	Brighton.

Nomination Certificate in favour of the following candidate was read for the first time and directed to be suspended in the Rooms of the Society in the usual manner :—

C. V. Adams, F.R.E.S.	Plymouth.
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Donations were reported from :—

Dr. Paul Vonwiller—

“ Lebendige Gewebelehre,” by P. Vonwiller.

Baillière, Tindall & Cox—

“ Experimental Catatonia,” by H. H. de Jong.

Mr. F. C. Grigg, F.R.M.S.—

36 Micro-slides showing plant cell development and structure.

Votes of thanks were accorded to the donors.

Paper.—The following communication was read and discussed :—

Dr. E. S. Horning, M.A., F.R.M.S.—

“ Experimental Induction of Prostatic Tumours in Mice.”

A vote of thanks was accorded to Dr. Horning for his communication.

Announcement.—The Secretary made the following announcement :—

The next meeting of the Biological Section will be held on Wednesday, April 3rd, 1946.

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, B.M.A. HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, APRIL 17TH, 1946, AT 5.30 P.M., DR. JAMES A. MURRAY, F.R.S., PAST-PRESIDENT, IN THE CHAIR.

The Minutes of the preceding meeting were read, confirmed, and signed by the Chairman.

New Fellow.—The following candidate was balloted for and duly elected an Ordinary Fellow of the Society :—

C. V. Adams, F.R.E.S.

Plymouth.

Nomination Certificate in favour of the following candidate was read for the first time and directed to be suspended in the Rooms of the Society in the usual manner :—

H. B. Harland.

Leeds.

Death.—The Chairman announced the regrettable loss to the Society, by death, of the following Fellow :—

J. A. Coultas.

Elected 1927.

Donation was reported from :—

Longmans Green & Co., Ltd.—

“ Quantitative Inorganic Microanalysis,” by R. Belcher and C. L. Wilson.

A vote of thanks was accorded to the donors.

New Council.—Nominations to serve on the new Council for election at a General Meeting of the Fellows to be held on Wednesday, May 15th, 1946, were read and approved.

Paper.—The following communication illustrated by a cinematograph film taken by phase-contrast photomicrography was read and discussed :—

Dr. W. F. Berg.—

“ Sperm Cell Development in the Grasshopper.”

A vote of thanks was accorded to Dr. Berg for his communication and exhibit.

Announcements.—The Secretary made the following announcements :—

The next meeting of the Biological Section will be held on Wednesday, May 1st, 1946.

A General Meeting of the Fellows will be held in the Rooms of the Society at 5.30 p.m., on Wednesday, May 15th, 1946, to receive the Report of the Council and the election of new Council.

The proceedings then terminated.

A GENERAL MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, B.M.A. HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, MAY 15TH, 1946, AT 5.30 P.M., DR. JAMES A. MURRAY, F.R.S., PAST-PRESIDENT, IN THE CHAIR.

The Minutes of the preceding meeting were read, confirmed, and signed by the Chairman.

New Fellow.—The following candidate was balloted for and duly elected an Ordinary Fellow of the Society :—

H. B. Harland.

Leeds.

Nomination Certificate in favour of the following candidate was read for the first time and directed to be suspended in the Rooms of the Society in the usual manner :—

A. H. Collinson.

Worthing.

Donation was reported from—

Dr. T. E. Wallis, F.R.M.S.—

“ Text Book of Pharmacognosy,” by T. E. Wallis.

Balance Sheet.—The Chairman called upon the Treasurer to submit the Audited Accounts and Financial Statement for the years 1942–1945.

TREASURER'S REPORT AND FINANCIAL STATEMENT FOR FOUR YEARS ENDING DECEMBER 31st, 1945

The last report issued for 1940–41 was published in Part 3 of the Journal, 1942, and the Fellows will be aware that during the War it has not been possible to prepare Accounts, and the Annual Meetings during that period were suspended under the authority granted by The Lord President of the Council (*vide Journ. Roy. Micr. Soc.*, 1941, LXI, 73).

There is now presented the Balance Sheet showing the state of the Society's finances at December 31st, 1945, together with Income and Expenditure Accounts for each of the four years ended December 31st, 1945.

Examining the Accounts it will be observed that although Subscriptions and Journal sales have come in fairly well, the accumulated amount owing is £1,181, and £657 has been reserved for doubtful debts on this Account.

During the period of four years there have been received seven Life Compounded Subscriptions, amounting to £219 10s. in the aggregate, and this has increased the amount at credit of the Life Compounded Subscription Account to £2,336 13s.

Due to repeated enemy action and other circumstances arising out of war conditions the Society's Journal has been unavoidably delayed in publication during the past two years, and a total provision of £960 has been made for publishing :—

	£	s.	d.
	960	0	0
Other specific reserves, including expenses of Centenary meeting and repairs to instruments, amount to	311	14	0
and accrued charges, together with amounts received in advance, aggregate	81	10	10
Making a total for sundry creditors, specific reserves and accrued charges of	1,353	4	10

The following are the summarized results for the four years to December 31st, 1945 :—

1942 Excess	...	...	...	...	...	...	...	...	£290 11 0
1943 „	...	...	...	...	...	...	...	...	390 5 5
1945 „	...	...	...	...	...	...	...	...	50 18 4
									731 14 9
1944 Deficiency	...	...	...	...	...	...	...	...	7 2 10
Net surplus over period	...	...	...	...	...	...	...	...	724 11 11
The deficiency brought forward as at December 31st, 1941, amounted to									167 8 8
Balance	...	...	..	...	...	...	...	...	557 3 3
and it is proposed, subject to the approval of the Council, to create a special Rehabilitation Reserve account of									500 0 0
leaving an amount at the credit of the account as at December 31st, 1945, to be carried forward of									£57 3 3

Income Tax deducted from investment income and recoverable by the Society has been credited in the Accounts up to and including the fiscal year 1941-42. Grants received towards expenses of publication of the Journal and donations during the four years amounted to £1,069.

There has been added to the Superannuation Reserve annual amounts of £100 for each of the four years and interest at the rate of $3\frac{1}{2}$ per cent. per annum, and this Reserve now amounts to £1,314 3s. 11d. In my report to the Fellows in 1936 I stated that the Council had made this provision to meet a retiring allowance in respect of the Society's Secretary. Dr. Tierney, I regret to say, is now retiring after 20 years' service including 6 years as Honorary Secretary, and the amount referred to above, subject to confirmation by the Council, will be used to purchase an annuity from the Atlas Assurance Company in his favour. I would like to acknowledge Dr. Tierney's great assistance to me especially during the last six years of exceptionally heavy war work.

I regret to have to report that the retiring Honorary Auditors, Messrs. Thomson McLintock & Co., do not wish to offer themselves for re-election. I would like to record my thanks to them for their kindness and help during their years of office ; their assistance and advice have been of the utmost help to the Society.

In submitting these Accounts I take my leave of the Treasurership. I have been Honorary Treasurer for 33 years, and regret that I have no longer the health to attend meetings. I would like to express my sincere thanks for the loyal support of the Fellows during my long term of office. I am sure my successor will have the same consideration.

C. F. HILL,
Honorary Treasurer.

Dr.		INCOME AND EXPENDITURE ACCOUNT FOR					
1941.		EXPENDITURE.					
£	s. d.			£	s. d.	£	s. d.
166	17 6	To Rent, Telephone and Insurance				167	2 11
350	0 0	„ Salaries				350	0 0
		„ Journal Expenditure—					
226	14 6	Printing		195	4 8		
170	18 11	Editing and Abstracting		165	0 6		
16	17 6	Illustrating		38	19 2		
9	16 1	Postages and Addressing		9	8 8		
		Reprints		22	9 5		
						431	2 5
		„ Other Expenditure—					
3	17 9	Library Books and Binding		5	1 10		
71	17 1	Stationery, Printing and Sundry Expenses		30	19 9		
4	13 1	Repairs and Renewals of Furniture		7	7 3		
						43	8 10
100	0 0	„ Reserve for Repairs to Instruments, etc.					
13	5 4	„ Depreciation on Furniture				11	18 10
		„ Superannuation Reserve—					
100	0 0	Annual Instalment		100	0 0		
22	18 6	Interest on balance of fund as at 1st January, 1942, at 3½% per annum		27	4 7		
						127	4 7
£1257	16 3					£1130	17 7
95	18 3	„ Excess of Income over Expenditure for year carried to Balance Sheet				290	11 0
£1353	14 6					£1421	8 7

Dr.		INCOME AND EXPENDITURE ACCOUNT FOR					
1942.		EXPENDITURE.					
£	s. d.			£	s. d.	£	s. d.
167	2 11	To Rent, Telephone and Insurance				167	5 1
350	0 0	„ Salaries				350	0 0
		„ Journal Expenditure—					
195	4 8	Printing		230	9 10		
165	0 6	Editing and Abstracting		163	7 6		
38	19 2	Illustrating		37	14 0		
9	8 8	Postages and Addressing		7	12 3		
22	9 5	Reprints		39	5 9		
						478	9 4
		„ Other Expenditure—					
5	1 10	Library Books and Binding		1	4 2		
30	19 9	Stationery, Printing, Postages and Sundry Expenses		29	16 9		
7	7 3	Repairs and Renewals of Furniture		8	17 6		
						39	18 5
11	18 10	„ Depreciation on Furniture				10	14 11
—		„ Reserve for Bad and Doubtful Debts				43	14 3
		„ Superannuation Reserve—					
100	0 0	Annual Instalment		100	0 0		
27	4 7	Interest on balance of fund at 1st January, 1943, at 3½% per annum		31	13 7		
						131	13 7
£1130	17 7					£1221	15 7
290	11 0	„ Excess of Income over Expenditure for year carried to Balance Sheet				390	5 5
£1421	8 7					£1612	1 0

THE YEAR ENDED 31ST DECEMBER, 1942.

Cr.

1941.		INCOME.		£	s.	d.	£	s.	d.
£	s.	d.							
			By Fellows Subscriptions for year	.	.	.	734	14	10
			„ Journal Subscriptions and Sales	.	.	.	277	14	6
944	8	3							1012 9 4
37	4	6	„ Journal Advertisements, less discounts	.	.	.		22	19 3
			„ Income from Investments—						
			Gross amount	.	.	.	147	14	7
118	16	0	Less : Income Tax deducted at source	.	.	.	34	17	4
									112 17 3
2	10	0	„ Bank Interest	.	.	.		2	10 0
			„ Grants towards cost of publication of Journal						
250	0	0	and Donation	.	.	.		205	5 0
—			„ Income Tax recovered	.	.	.		64	7 1
15	9		„ Other Receipts	.	.	.		1	0 8

£1353 14 6£1421 8 7

THE YEAR ENDED 31ST DECEMBER, 1943.

Cr.

1942.		INCOME.		£	s.	d.	£	s.	d.
£	s.	d.							
734	14	10	By Fellows Subscriptions	.	.	.	767	6	3
277	14	6	„ Journal Subscriptions and Sales	.	.	.	372	14	1
									1140 0 4
22	19	3	„ Journal Advertisements less discounts	.	.	.		41	18 6
			„ Income from Investments—						
			Gross amount	.	.	.	155	9	2
			Less : Income Tax deducted at source	.	.	.	37	2	2
112	17	3							118 7 0
2	10	0	„ Bank Interest	.	.	.		2	10 0
			„ Grant received towards cost of publication of						
205	5	0	Journal, and Donation	.	.	.		307	17 9
64	7	1	„ Income Tax recovered	.	.	.		—	
1	0	8	„ Other Receipts	.	.	.		1	7 5

£1421 8 7£1612 1 0

Dr.			INCOME AND EXPENDITURE ACCOUNT FOR					
1943.			EXPENDITURE.					
£	s.	d.				£	s.	d.
167	5	1	To Rent, Telephone and Insurance	.	.			166 9 10
350	0	0	„ Salaries	.	.			350 0 0
			„ Journal Expenditure—					
230	9	10	Printing—provision	.	.	400	0	0
163	7	6	Editing and Abstracting	.	.	159	0	0
37	14	0	Illustrating	.	.	45	6	7
7	12	3	Postages and Addressing	.	.	10	0	0
39	5	9	Reprints	.	.	40	0	0
								654 6 7
			„ Other Expenditure—					
1	4	2	Library Books and Binding	.	.	3	17	9
29	16	9	Stationery, Printing, Postages and Sundry	.	.			
8	17	6	Expenses	.	.	16	17	9
			Repairs and Renewals of Furniture, etc.	.	.	9	15	9
								30 11 3
10	14	11	„ Depreciation on Furniture	.	.			9 13 5
43	14	3	„ Reserve for Bad and Doubtful Debts	.	.			128 2 0
			„ Superannuation Reserve—					
100	0	0	Annual Instalment	.	.	100	0	0
			Interest on balance of fund at 1st January,					
			1944, at 3½% per annum	.	.	36	5	10
								136 5 10
31	13	7						
£1221	15	7						£1475 8 11

Dr.			INCOME AND EXPENDITURE ACCOUNT FOR					
1944.			EXPENDITURE.					
£	s.	d.				£	s.	d.
166	9	10	To Rent, Telephone and Insurance	.	.			166 17 4
350	0	0	„ Salaries	.	.			350 0 0
			„ Journal Expenditure—					
400	0	0	Printing—provision	.	.	400	0	0
159	0	0	Editing and Abstracting	.	.	159	0	0
45	6	7	Illustrating	.	.	35	4	11
10	0	0	Postages and Addressing	.	.	10	0	0
40	0	0	Reprints	.	.	40	0	0
								644 4 11
			„ Other Expenditure—					
3	17	9	Library Books and Binding	.	.	52	11	9
16	17	9	Stationery, Printing, Postages and Sundry	.	.			
9	15	9	Expenses	.	.	32	10	5
			Repairs and Renewals of Furniture	.	.	9	18	1
								95 0 3
9	13	5	„ Depreciation on Furniture	.	.			8 14 1
128	2	0	„ Reserve for Bad and Doubtful Debts	.	.			206 5 0
			„ Superannuation Reserve—					
100	0	0	Annual Instalment	.	.	100	0	0
			Interest on balance of fund at 1st January,					
			1945, at 3½% per annum	.	.	41	1	2
								141 1 2
1475	8	11						1612 2 9
			„ Excess of Income over Expenditure for year					
			carried to Balance Sheet	.	.			50 18 4
£1468	6	1						£1663 1 1

THE YEAR ENDED 31st DECEMBER, 1944.

Cr.

1943.			INCOME.						
£	s.	d.		£	s.	d.	£	s.	d.
767	6	3	By Fellows' Subscriptions	785	16	11			
372	14	1	„ Journal Subscriptions and Sales	231	15	1			
							1017	12	0
41	18	6	„ Journal Advertisements, less discount				20	7	3
			„ Income from Investments—						
			Gross amount	172	11	6			
			Less : Income Tax deducted at source	45	13	4			
118	7	0					126	18	2
2	10	0	„ Bank Interest				2	10	2
			„ Grant received towards expenses of publication						
307	17	9	of Journal				300	0	0
1	7	5	„ Other Receipts				18	6	
1612	1	0					1468	6	1
			„ Excess of Expenditure over Income for year,						
390	5	5	carried to Balance Sheet				7	2	10
<u>£1221 15 7</u>							<u>£1475 8 11</u>		

THE YEAR ENDED 31st DECEMBER, 1945.

Cr.

1944.			INCOME.						
£	s.	d.		£	s.	d.	£	s.	d.
785	16	11	By Fellows' Subscriptions	835	4	6			
231	15	1	„ Journal Subscriptions and Sales	417	1	5			
							1252	5	11
20	7	3	„ Journal Advertisements, less discount				19	4	9
			„ Income from Investments—						
			Gross amount	181	9	2			
			Less : Income Tax deducted at source	50	2	2			
126	18	2					131	7	0
2	10	2	„ Bank Interest				2	9	11
300	0	0	„ Grant received towards expenses of publication						
			of Journal, and Donation				256	6	0
18	6		„ Other Receipts				1	7	6
£1468 6 1							£1663 1 1		

BALANCE SHEET AS

	LIABILITIES.	£	s.	d.	£	s.	d.	£	s.	d.
I. Capital—										
Being (a)	Life Compounded Subscriptions from 1st January, 1877 to 31st December, 1944	2305	3	0						
	Add: Amount received during 1945	31	10	0	2336	13	0			
(b)	Quekett Memorial Fund				100	0	0			
(c)	Mortimer Bequest				45	0	0			
(d)	A. N. Disney Bequest				100	0	0			
(e)	Amount received in respect of Sales of Books and Catalogues from the Library (surplus to the Society's requirements) to 31st December, 1944	332	18	3						
	Add: Sales during 1945	6	5	6	339	3	9			
(f)	Admission Fees to 31st Decem- ber, 1944	615	6	0						
	Add: Fees during 1945	73	10	0	688	16	0			
(g)	A. Gandolfi Hornyold Bequest				100	0	0			
(h)	F. Balfour-Browne— Gift				100	0	0			
					3809	12	9			
	Less: Amount transferred to Super- annuation Reserve in respect of year ended 31st December, 1935				100	0	0			
								3709	12	9
II. Sundry Creditors, Specific Reserves and Accrued Charges—										
	Subscriptions paid in advance				44	17	6			
	Journal Subscriptions paid in advance				34	4	0			
	Accrued Charges				2	9	4			
	Reserve for expenses in connection with the Centenary Meeting				211	14	0			
	Reserve for Repairs to Instruments				100	0	0			
	Reserve for cost of printing Journal, Illustrating, Postages and Reprints				960	0	0			
								1353	4	10
	Forward							£5062	17	7

AT 31ST DECEMBER, 1945.

	ASSETS.	£	s.	d.	£	s.	d.
I. Furniture and Equipment—							
As at 31st December, 1944		87	0	9			
Less : Depreciation at 10% per annum		8	14	1			
					78	6	8
II. Investments at Cost, less amount written off—							
					4415	10	3
£400 London and North Eastern Railway Co. 3% Debenture Stock.							
£500 Nottingham Corporation 3% Irredeemable Debenture Stock.							
£150 Metropolitan Water Board "B" Stock.							
£612 London Midland and Scottish Railway Co. 4% Preference Stock.							
£200 New South Wales 5½% Loan, 1917–57.							
£100 3% Conversion Loan, 1948–53.							
£421 1s. 3½% War Stock (Registered).							
£400 3½% War Stock (Inscribed).							
£300 Birmingham Corporation 3¼% Redeemable Stock, 1957–62.							
£400 3% Defence Bonds.							
£250 3% Savings Bonds.							
£600 3% Defence Bonds.							
£1200 3% Savings Bonds.							
<i>Note.</i> —The market value of the above investments at 31st December, 1945, was approximately £5390 9s. 6d. In accordance with the Resolution of the Council dated 21st October, 1942, £900 3% Defence Bonds was earmarked in respect of the Superannuation Reserve.							
III. Sundry Debtors—							
Subscriptions and Journal Sales		1181	13	0			
Less : Reserve for Doubtful Debts		657	9	9			
					524	3	3
IV. Cash at Bank and in Hand—							
National Provincial Bank, Limited—							
Deposit Account		500	0	0			
Current Account		1410	8	2			
In Hand		5	16	5			
					1916	4	7
Forward							
					£6934	4	9

BALANCE SHEET AS

LIABILITIES—*continued*.

	£	s.	d.	£	s.	d.	£	s.	d.
Forward							5062	17	7
III. <i>Superannuation Reserve</i> —									
Annual Instalments and Interest as at 31st December, 1944				1173	2	9			
Add : Instalment for year ended 31st December, 1945	100	0	0						
Interest at 3½% per annum	41	1	2						
				141	1	2			
							1314	3	11
IV. <i>Rehabilitation Reserve</i> —									
Amount transferred from Income and Expenditure Account, subject to approval of Council							500	0	0
V. <i>Income and Expenditure Account</i> —									
Balance at Credit at 31st December, 1944				506	4	11			
Add : Excess of Income over Expenditure for year to date				50	18	4			
				557	3	3			
Less : Amount appropriated to Rehabilitation Reserve, as above	500	0	0				57	3	3
							£6934	4	9

C. F. HILL, *Hon. Treasurer*.

London, 29th March, 1946. We have examined the Books and Accounts of the Royal Microscopical Society for the year ended 31st December, 1945, and have prepared therefrom the above Balance Sheet.

AT 31st DECEMBER, 1945.

ASSETS— <i>continued</i>	£	s.	d.
Forward . . .	£6934	4	9

£6934 4 9

No account has been taken of the value of the Society's Library, Stock of Journals and Collection of Instruments (values for insurance together with the furniture and equipment at £7,000). Subject thereto, the above Balance Sheet is, in our opinion, properly drawn up so as to exhibit a true and correct view of the state of the Society's affairs at 31st December, 1945, according to the best of our information and the explanations given to us.

(Signed) THOMSON McLINTOCK & CO.,

Chartered Accountants, Honorary Auditors.

2, Surrey Street, London, W.C.2.

STATEMENT OF MEMBERSHIP AT DECEMBER 31st, 1945.

The number of Fellows on the Roll of the Society at December 31st, 1941, was	...	...	...	...	...	...	...	...	418
and there have been the following changes in membership during the four years ended December 31st, 1945 :—									
<i>Add</i> : Fellows elected	...	...	...	...	...	...	...	95	
Fellows reinstated	...	...	...	...	...	...	...	2	
								—	97
									515
<i>Deduct</i> : Fellows resigned or removed	...	...	...	...	...	...	...	15	
Fellows deceased	...	...	...	...	...	...	...	26	
								—	41
Number of Fellows on the Roll at December 31st, 1945	...	...							474
Made up as follows :—									
(a) Ordinary Fellows	...	...	...	...	...	...	...	436	
(b) Compounded Fellows	...	...	...	...	...	...	...	25	
(c) Honorary Fellows	...	...	...	...	...	...	...	13	
								—	474

ONE HUNDRED AND SIXTH ANNUAL REPORT.

REPORT OF THE COUNCIL FOR THE YEARS 1944–1945.

The Report of the Council for the years 1944–1945 was read by the Secretary as follows :—

With the victorious end for the Allies of the Second World War after six years of bitter and unrelenting struggle in which London was the central target, the Council is glad to report that despite the grim difficulties and constant hazards during which the work of the Society was continued and maintained without intermission, no irreparable loss has been suffered by the Society in respect of its historical collections, library, or other property, with the exception of the regrettable loss of the whole of the Society's current books of accounts, registers and other documents and records, and certain Journal matter referred to later, which were totally destroyed by enemy action in the devastating burning of London in May, 1941.

At that period and since, when intensive bombing, both high-explosive and incendiary, became a menacing and continuous anxiety and it appeared that nothing that the Council could do would ensure the avoidance of risk, it was nevertheless resolved that the historical instruments in the Society's possession should be packed and distributed as far as possible in widely scattered outlying districts less liable to hostile

attack. The Council now reports that the whole of the collection is in process of re-assembly and is practically undamaged, except in the case of one fragile instrument which suffered minor damage from bomb blast. The thanks of the Society have been duly conveyed to those with whom the instruments were deposited for safe custody.

Throughout the whole of this anxious period, during which considerable damage was suffered at the Society's premises, a very heavy responsibility devolved upon the Secretary, Dr. C. Tierney, for the care and maintenance of the Society's activities and affairs and for the safe custodianship of its property. The unremitting strain imposed upon him by the circumstances, and discharged with exemplary devotion, has not been without effect upon his health; and in view of this and of his advancing years he now feels obliged to relinquish his office as Secretary to the Society and has urged upon the Council the acceptance of his retirement as soon as may be agreeable and convenient in the ensuing year.

This is not the occasion on which to refer at length to Dr. Tierney's long record of outstanding service to the Society. But the Council desires on its own behalf and on behalf of the Fellows not to let this opportunity pass without placing on record their high appreciation of his devoted and able administration, and their grateful thanks to him for his unfailing interest in the Society's welfare and maintenance throughout many eventful years. It is with great reluctance that the Council is now obliged to accede to his request to be released from his executive duties, and the Fellows will join with them in wishing him well in his retirement, and in the earnest hope that his experienced guidance and advice will continue to be available to them for many years to come.

PRESIDENT.

OFFICERS AND MEMBERS OF COUNCIL.

In accordance with the by-laws and the direction granted by the Lord President of the Council (*Journ. Roy. Micr. Soc.*, 1941, LXI, 73) the following have continued to serve in office :—

PRESIDENT,

Mr. J. E. Barnard, F.R.S.

VICE-PRESIDENTS,

Dr. R. S. Clay.

Dr. G. M. Findlay, C.B.E.

Mr. M. T. Denne, O.B.E.

Mr. D. J. Scourfield, I.S.O.

HON. TREASURER,

Mr. C. F. Hill.

HON. SECRETARY,

Dr. G. M. Findlay, C.B.E.

ORDINARY MEMBERS OF COUNCIL,

Mr. R. J. Bracey.

Dr. J. E. McCartney.

Dr. H. M. Carleton.

Dr. J. A. Murray, F.R.S.

Prof. R. Ruggles Gates, F.R.S.

Mr. J. H. Pledge.

Mr. N. Ingram Hendey.

Dr. T. E. Wallis.

Dr. R. J. Ludford.

Mr. H. Wrighton.

Mr. S. R. Wycherley.

HON. EDITOR,
Dr. G. M. Findlay, *C.B.E.*

HON. CURATOR OF INSTRUMENTS,
Mr. M. T. Denne, *O.B.E.*

HON. CURATOR OF SLIDES,
Mr. N. Ingram Hendey.

SECRETARY AND LIBRARIAN,
Dr. C. Tierney.

At the Special General Meeting now assembled the Fellows will be requested to elect a new Council to serve during the ensuing year.

FELLOWS.

Since the last report the Council deplores the loss the Society has sustained, by death, of the following Fellows :—

Mr. Conrad Beck, Past-President.	Elected 1885.
Prof. D. M. Blair.	„ 1928.
Mr. A. J. Bowtell.	„ 1921.
Mr. F. Chapman, Hon. Fellow 1929.	„ 1892.
Sir John Bretland Farmer, Hon Fellow.	„ 1930.
Rev. Dingley P. Fuge.	„ 1934.
Mr. J. A. Long.	„ 1927.
Mr. E. G. MacLeod.	„ 1925.
Mr. H. Taverner.	„ 1900.
Mr. C. S. Todd.	„ 1938.

Sixty-two candidates have been elected Ordinary Fellows of the Society, and one has been reinstated to the Roll of Fellowship. Ten have resigned.

MEETINGS.

With the relaxing of the official black-out regulations normal monthly meetings of Fellows were resumed in October last. Transport difficulties, however, continued to affect the attendance, especially of those Fellows living at a distance from London. With the improved travelling facilities now contemplated and the return of many of the Fellows from service and other war-time engagements, increased attendance at the Society's meetings is confidently expected.

Meetings of the Biological Section have also been resumed under the secretaryship of Mr. N. Ingram Hendey in succession to Mr. D. J. Scourfield.

JOURNAL.

In addition to the destruction previously mentioned the Council regrets to report that the Society's Journal has not escaped unfortunate delay in publication consequent upon repeated enemy action and other circumstances beyond the Council's control. With the total destruction of the London works of the Society's printers and also those of the Society's block-makers, resulting in loss of type, blocks, paper supplies and other Journal matter, the office was involved in considerable correspondence and delay

affecting the issue of the Journal then in hand and subsequent issues. This matter is now again in hand and receiving urgent consideration. It is contemplated that the issues now in press will shortly be available for distribution.

LIBRARY, INSTRUMENT AND SLIDE COLLECTIONS.

Increased use continues to be made of the Society's Library and several works not in the Society's possession have been obtained for the use of the Fellows through the National Central Library under reciprocal arrangements. Since the last report forty-four volumes have been presented to the Library and the thanks of the Fellows have been duly conveyed to the donors.

In addition the Society has received from the Executors of the late Mr. C. D. Soar a valued collection of original drawings, note-books and miscellaneous reprints, mainly on the *Hydracharina*, which have been gratefully acknowledged.

The Society's instrument and slide collections have also been further enriched by the following accessions :—

From the Exors. of the estate of the late Mr. F. E. D. Acland—

A pocket microscope with accessories in case, by Nachet.

Mr. C. Lees Curties, F.R.M.S.—

An early type of Linen testing glass.

Mr. J. J. Grover, F.R.M.S.—

A $\frac{1}{4}$ -inch objective by Andrew Ross with correction collar, dated 1852.

Dr. L. P. Clarke, F.R.M.S.—

A portable microscope of the Moginie type, by Swift, with accessories in case.

Mr. J. E. Barnard, F.R.S., P.R.M.S.—

A $12\frac{1}{2}$ in. $\times$ 9 in. sheet of transparent silvered glass 2.6/3.2 mm., by Pilkington Bros.

Due acknowledgement has been conveyed to the above donors for these valued accessions.

In submitting this report upon the Society's affairs at the end of a most anxious period which the Society has successfully survived, the Council is not without cognizance of the difficulties that prevailing abnormal conditions still impose. With the resumption of the Society's full activities and ordinary monthly meetings they have confidence in relying upon the Fellows for their united co-operation and support to ensure that the long and honoured traditions of service that have distinguished this Society in the study and advancement of microscopical science shall continue to be maintained.

On the motion of Mr. J. Smiles, seconded by Major Maxwell Knight, it was unanimously resolved—

“That the Report of the Council and the Audited Accounts be received and adopted.”

It was further resolved on the motion of Mr. F. C. Grigg, seconded by Mr. A. C. Cramp—

“That a hearty vote of thanks be tendered to the Officers and Members of Council for their continued services to the Society during the war years.”

New Council.—The Chairman appointed Mr. M. P. Young and Mr. J. K. Letts to act as Scrutineers of the Ballot for the election of Officers and Members of Council for the ensuing year; and subsequently, upon receipt of the Scrutineers' report declared the result of the ballot as follows—

President.—*James A. Murray, M.D., F.R.S.

Vice-Presidents.—*Mr. J. E. Barnard, F.R.S.; Dr. G. M. Findlay, C.B.E.; *Mr. C. F. Hill; *Dr. T. E. Wallis.

Hon. Treasurer.—*Mr. S. R. Wycherley.

Hon. Secretaries.—*Dr. R. J. Ludford; *Dr. H. Moore.

Ordinary Members of Council.—*Mr. F. D. Armitage; *Dr. L. P. Clarke; *Dr. F. Greenshields; *Mr. F. C. Grigg; Mr. N. Ingram Hendey; *Major Maxwell Knight; Dr. J. E. McCartney; *Mr. Chetwynd Palmer; *Mr. R. Ross, M.A.; *Mr. D. J. Scourfield, I.S.O.; *Mr. E. Wilfred Taylor; *Mr. J. M. Watson, A.R.C.S.

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(Those marked with an asterisk have not held during the war years the office to which they have now been elected.)

On the motion of the Chairman a hearty vote of thanks was accorded to the Scrutineers for their services.

Paper.—The following communication was read and discussed:—

Dr. T. E. Wallis, F.R.M.S.—

“The Microscopy of Plant Hairs.”

A vote of thanks was accorded to Dr. Wallis for his communication.

Announcements.—The Secretary made the following announcements:—

The next Ordinary Meeting will be held on Wednesday, October 16th, 1946.

The next meeting of the Biological Section will be held on Wednesday, November 6th, 1946.

The Rooms of the Society will be closed for the Summer Vacation from August 5th to August 31st, 1946.

The Proceedings then terminated.

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JOURNAL
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TRANSACTIONS OF THE SOCIETY.

I.—ANATOMICAL VARIATION IN RICE *ORYZA SATIVA* LINN.

By S. HEDAYETULLAH, M.Sc.(Cal.), Ph.D.(Lond.)

and

A. K. CHAKRAVORTY, M.Sc.(Cal.).

(Central Agricultural Research Station, Dacca, Bengal.)

ONE PLATE AND TABLE.

INTRODUCTION.

VERNON (1942) emphasized the importance of anatomy in taxonomic and phylogenetic studies of the monocotyledons in special cases where floral structure cannot be interpreted with certainty. Suzuki (1936) found correlation of fungus diseases with certain anatomical characters of the rice plant. It has been observed by the authors (1941) that the strength of straw very much depends on the mechanical tissues occurring in the stem of the genus *Oryza*. Hamilton (1941) studied the relationship of certain anatomical characters to lodging in oats, and found out that the number of vascular bundles and their size were much greater in strong strawed species.

Anatomical characters in the rice plants may be utilized in connection with the classification of different varieties and selection of disease resistant and non-lodging types. Before using the anatomical characters for classification and breeding purposes it is necessary that the variation of such characters should be studied. Variations of anatomical characters among different varieties and the tillers of the same plant will be dealt with in the present paper.

MATERIAL AND METHOD.

Samples of the following transplanted rice varieties, viz. : *Jhanji 34*, *Dharial*, *Badkalamkati 65*, *Latisail*, *Patnai 23*, and *Bhasamanik* were all collected from the experimental plots of Rice Research Station, Chinsura in 1939. In each case an entire plant was uprooted during the time of flowering and the number of tillers were counted, the main tiller (the longest tiller of the plant) was numbered as 1 (one) and others serially as 2, 3, 4, etc., at random.

The tillers were then cut into internodes. The visible internode just above the ground level was marked as the first internode. The materials were preserved in 4 p.c. formalin solution.

Variety.	Total number of tiller in the plant.	Tiller examined.	Date of ear emergence.	Date of sampling.	Date of examination.
<i>Jhanji 34</i>	15	1 2 3	8.9.39	8.9.39	1.4.40 to 23.7.41
<i>Dharial</i>	6	1 2 3	10.8.39	8.9.39	
<i>Badkalamkati 65</i> ...	14	1 2 3	20.9.39	23.9.39	
<i>Latisail</i>	11	1 2 3	20.10.39	23.11.39	
<i>Patnai 23</i>	7	1 2 3	23.10.39	„	
<i>Bhasamanik</i>	9	1 2 3	„	„	

Materials were examined both in section and maceration.

(1) *Sections* were cut in hand microtoms 10–15 microns thick and stained in safranin-hæmatoxylin, light-green safranin, and iodine green acid fuchsin combinations. Permanent slides were made after proper dehydration and mounting in Canada balsam. Microscopic examination of the sections were made by making temporary mounts on a glass slide with a drop of glycerine after transferring the sections directly from water. To distinguish between the lignified and non-lignified tissues a drop of safranin was added. Sections were then projected with the help of a camera lucida on a piece of paper fixed on the table and measurements were recorded with a centimetre scale. They were then adjusted with the stage micrometer by projecting the same as above.

(2) *Macerations* were done by cutting the internodes into pieces with scalpel and keeping them in 10 p.c. chromic and nitric acids for 20–24 hours at room temperature in summer, and for 22 hours in the incubator at 36° C. in the winter

season. The macerated materials were then thoroughly washed in running water and made ready for examination. Permanent preparations were made after proper dehydration and staining with safranin. Microscopic examinations were made after taking a little material on a glass slide adding a drop of glycerine and safranin, and covering by means of cover-slips. Measurements were taken with the help of eye-piece micrometer adjusted by means of stage micrometer.

Both the sections and macerated materials were kept in glycerin alcohol solution (10 p.c. glycerine in 50 p.c. alcohol). They remained fresh for about three months in the dry season. Measurements were taken of the diameter of the internodes and length of the internodes by means of a centimetre scale. The number of layers of cells with their thickness in the sub-epidermal fibrous cylinder, the number of vascular bundles, their tangential and radial diameters in the sub-epidermal and sub-cortical mechanical rings, total number of vascular bundles and sub-cortical air chambers were determined in the section. The length and breadth of fibres and vessels were measured in macerated material. For analysing the data statistically six countings and six measurements of the number of layers of cells in the sub-epidermal fibrous cylinder and their thickness, the tangential and the radial diameters of the vascular bundles in the sub-epidermal and in the sub-cortical rings were made from each section. Similarly twenty-five measurements for fibre length and breadth and ten measurements for vessel length and breadth have been made from the macerated materials. The mean value with their standard errors have been recorded.

Length and breadth of fibres were measured from different fibres, whereas vessel length and breadth were measured from the same vessel. Breadth of fibres was not equal throughout the length. Measurements were therefore taken in the region of maximum breadth. In measuring the vessel length we have followed the directions of Chalk and Chattaway (1934).

ANATOMICAL DESCRIPTION OF THE VARIETIES.

1. *Jhanji 34.*

Sub-epidermal fibrous cylinder : 3 cells wide, $7.14 \pm 0.40\mu$ – $7.86 \pm 0.31\mu$ in thickness.

Isolated fibres : very short $452.6 \pm 18.4\mu$ – $838.1 \pm 48.7\mu$ in length, $12.54 \pm 1.09\mu$ – $15.27 \pm 0.81\mu$ in breadth, fibre tips pointed or curved or of other shapes. Septate or non-septate, with closed lumen, no inclusion was present, spirally pitted with slit-shaped pits.

Parenchyma : of various shapes and sizes, 2–3 rows of parenchymatous cells below the sub-epidermal fibrous cylinder in some cases thick walled.

Fibro-vascular bundles in the sub-epidermal ring : 21–23 in number, are free from the mechanical tissues of sub-epidermal fibrous cylinder. Radial diameter of fibro-vascular bundles was $120.53 \pm 6.73\mu$ – $142.7 \pm 5.30\mu$ and tangential diameter $126.06 \pm 12.73\mu$ – $135.53 \pm 11.20\mu$. Extra number of fibro-vascular bundles is 3, which may remain scattered or fused with other bundles in the ring.

Fibro-vascular bundles in the sub-cortical ring : 21–23 in number, occurring at regular intervals in the ring below each sub-epidermal fibro-vascular bundles

(fig. 2). Radial diameter of bundles was $144.40 \pm 11.33\mu$ – $162.73 \pm 10.66\mu$ and tangential diameter $119.40 \pm 6.33\mu$ – $146.66 \pm 9.66\mu$. Extra number of bundles may be 3 or 4 which remain scattered in the ring.

Total number of fibro-vascular bundles in the stem : 46–50.

Vessel members : $111.0 \pm 7.5\mu$ – $296.7 \pm 24.3\mu$ in length and $42.6 \pm 2.2\mu$ – $51.0 \pm 2.7\mu$ in breadth. Walls with simple pits, perforation plates empty or hollow, slightly oblique.

Number of sub-cortical air chambers : 21–23.

2. *Dhural.*

Sub-epidermal fibrous cylinder : 2–4 cells wide, $6.30 \pm 0.17\mu$ – 7.26 ± 0.43 in thickness.

Isolated fibres : very short $636.8 \pm 44.7\mu$ – $689.4 \pm 47.3\mu$ in length, $9.54 \pm 0.54\mu$ – $12.54 \pm 0.81\mu$ in breadth, fibre tips pointed or curved, non-septate, no inclusion was present. Wavy outlines in the walls of some fibres were found (fig. 6).

Parenchyma : of various geometrical shapes and sizes.

Fibro-vascular bundles in the sub-epidermal ring : 20–25 in number embedded within the mechanical tissues of sub-epidermal fibrous cylinder (fig. 4). Radial diameter of fibro-vascular bundles $108.33 \pm 6.46\mu$ – $191.06 \pm 9.80\mu$, tangential diameter $104.40 \pm 6.06\mu$ – $140.00 \pm 8.66\mu$. Extra number of fibro-vascular bundles may be up to 8 in number within the sub-epidermal ring. Three bundles may remain scattered or fused with each other.

Fibro-vascular bundles in the sub-cortical ring : 20–25 in number occurred at regular intervals in the ring, below each sub-epidermal fibro-vascular bundles. Radial diameter of fibro-vascular bundles $149.40 \pm 11.66\mu$ – $217.20 \pm 14.06\mu$, tangential diameter $110.53 \pm 5.86\mu$ – $175.53 \pm 8.66\mu$. Extra number of vascular bundles are up to 10 in number and they remain scattered in the ring.

Total number of vascular bundles in the stem : 56–58.

Vessel members : $119.1 \pm 9.9\mu$ – $280.5 \pm 17.4\mu$ in length and $41.4 \pm 2.7\mu$ – $51.3 \pm 3.8\mu$ in breadth. Walls with simple pits, perforation plate hollow, inclination of perforation plate oblique.

Number of sub-cortical air chambers : 20–25.

3. *Badkalamkati 65.*

Sub-epidermal fibrous cylinder : 3–6 cells wide, $7.86 \pm 40\mu$ – $10.51 \pm 0.94\mu$ in thickness.

Isolated fibres : very short, $618.4 \pm 39.4\mu$ – $852.6 \pm 43.4\mu$ in length $11.18 \pm 0.27\mu$ in breadth, pointed or curved at the tip, few with wavy outlines in the walls.

Parenchyma : of all shapes and sizes.

Fibro-vascular bundles in the sub-epidermal ring : 24–32 in number may be attached or free with the mechanical tissues of sub-epidermal fibrous cylinder (fig. 1). Radial diameter $100.00 \pm 3.73\mu$ – $114.40 \pm 7.00\mu$ and the tangential diameter $131.06 \pm 9.06\mu$ – $156.66 \pm 10.86\mu$. Extra number of fibro-vascular bundles up to 3 in number occur in the ring, which remain scattered.

Fibro-vascular bundles in the sub-cortical ring : 24–32 in number occurring at regular intervals in the ring below each sub-epidermal fibro-vascular bundles. Radial diameter of the bundles is $161.66 \pm 6.60\mu$ – $221.06 \pm 5.20\mu$, and the tangential diameter $142.73 \pm 6.73\mu$ – $186.06 \pm 18.26\mu$. Extra number of fibro-vascular bundles is up to 3 in number, and they remain scattered in the ring.

Total number of vascular bundles in the stem : 53–64.

Vessel members : $177.6 \pm 16.5\mu$ – $289.5 \pm 22.5\mu$ in length and $47.7 \pm 3.8\mu$ – $51.9 \pm 3.3\mu$ in breadth. Walls with simple pits, perforation plates straight or oblique.

Number of sub-cortical air chambers : 24–32.

4. *Latisail.*

Sub-epidermal fibrous cylinder : 2 cells wide, 5.58 ± 0.33 – $6.90 \pm 0.34\mu$ in thickness.

Isolated fibres : very short, $435.5 \pm 25.0\mu$ – $561.3 \pm 28.9\mu$ in length, 10.36 ± 0.54 – $12.27 \pm 0.54\mu$ in breadth, pointed or curved at the tips or of other shapes, non-septate with closed lumen, spirally pitted with slit-shaped pits.

Parenchyma : with all shapes and sizes.

Fibro-vascular bundles in the sub-epidermal ring : 25–27 in number free from the mechanical tissues of sub-epidermal fibrous cylinder. Radial diameter $84.40 \pm 5.80\mu$ – $103 \pm 6.93\mu$ and the tangential diameter $109.40 \pm 5.33\mu$ – $137.73 \pm 4.53\mu$. Extra number of vascular bundles is up to 3 in number, may remain scattered or fused with other fibro-vascular bundles (fig. 3).

Fibro-vascular bundles in the sub-cortical ring : 23–39 in number, occurred at regular intervals below each sub-epidermal fibro-vascular bundle, with the exception of few bundles. Radial diameter of the bundles $124.40 \pm 8.40\mu$ – $163.86 \pm 5.73\mu$ and the tangential diameter $107.73 \pm 5.60\mu$ – $135.00 \pm 3.00\mu$. Extra number of vascular bundles may be up to 9 in number which remain scattered in the ring.

Total number of vascular bundles in the stem : 52–62.

Vessel members : $136.8 \pm 9.6\mu$ – $217.2 \pm 24.3\mu$ in length and $26.7 \pm 3.0\mu$ – $46.2 \pm 2.2\mu$ in breadth, walls with simple pits, perforation plate hollow, elliptical, inclination of perforation plates slightly oblique to oblique.

Number of sub-cortical air chambers : 21–26.

5. *Patnai 23.*

Sub-epidermal fibrous cylinder : 2–5 cells wide, $4.76 \pm 0.34\mu$ – $7.14 \pm 0.47\mu$ in thickness.

Isolated fibres : very short $469.7 \pm 48.7\mu$ – $868.4 \pm 34.2\mu$ in length, $7.63 \pm 0.27\mu$ – $12.00 \pm 0.54\mu$ in breadth, pointed or curved at the tip, non-septate or few septate (with one septa) no inclusion was present.

Parenchyma : squarish, or of other geometrical shapes.

Fibro-vascular bundles in the sub-epidermal ring : 18–22 in number, they may be embedded, attached or free with the mechanical tissues of the sub-epidermal

fibrous cylinder. Radial diameter $83.33 \pm 4.06\mu$ – $97.20 \pm 3.66\mu$ tangential diameter $107.20 \pm 5.33\mu$ – $132.20 \pm 6.00\mu$. Extra number of fibro-vascular bundles may be up to 5 which remain scattered or attached with other bundles in the ring.

Fibro-vascular bundles in the sub-cortical ring : 18–22 in number occurred at regular intervals below each sub-epidermal fibro-vascular bundles. Radial diameter $137.73 \pm 4.53\mu$ – $166.66 \pm 7.80\mu$ and the tangential diameter $118.86 \pm 8.00\mu$ – $125.53 \pm 4.53\mu$. Extra number of fibro-vascular bundles is up to 7 in number, which remain scattered in the ring.

Total number of vascular bundles in the stem : 39–49.

Vessel members : $136.2 \pm 17.7\mu$ – $339.9 \pm 27.3\mu$ in length, $35.4 \pm 1.6\mu$ – $39.2 \pm 4.5\mu$ in breadth, walls with simple pits, perforation plate hollow, inclination of perforation plate, straight, slightly oblique to oblique.

Number of sub-cortical air chambers : 18–22.

6. *Bhasamanik.*

Sub-epidermal fibrous cylinder : 3–5 cells wide, $7.26 \pm 0.57\mu$ – $8.57 \pm 0.34\mu$ in thickness.

Isolated fibres : very short, $315.8 \pm 7.9\mu$ – $1040.8 \pm 19.7\mu$ in length, $12.00 \pm 1.90\mu$ – $13.63 \pm 0.81\mu$ in breadth, pointed or curved at the tip, non-septate or few septate (septa one or two) no inclusion was present.

Parenchyma : of various geometrical shapes and sizes.

Fibro-vascular bundles in the sub-epidermal ring : 22–26 in number, they may be attached or free with the mechanical tissues of the sub-epidermal fibrous cylinder. Radial diameter $98.33 \pm 3.00\mu$ – $126.66 \pm 12.73\mu$ and the tangential diameter $147.20 \pm 6.60\mu$ – $165.53 \pm 9.33\mu$. Extra number of fibro-vascular bundles is nil.

Fibro-vascular bundles in the sub-cortical ring : 22–26 in number, occurred at regular intervals below each sub-epidermal fibro-vascular bundles. Radial diameter $154.40 \pm 3.46\mu$ – $168.86 \pm 3.20\mu$ and the tangential diameter $153.00 \pm 11.06\mu$ – $161.66 \pm 5.26\mu$. Extra number of fibro-vascular bundles is up to 3 which remain scattered in the ring.

Total number of vascular bundles in the stem : 47–52.

Vessel members : $90.0 \pm 1.6\mu$ – $309.6 \pm 20.7\mu$ in length, $42.9 \pm 2.7\mu$ – $54.3 \pm 4.5\mu$ in breadth, walls with simple pits, perforation plate hollow, inclination of perforation plate straight or oblique.

Number of sub-cortical air chambers : 22–26.

DISCUSSION.

Varietal Variation.—*Badkalamkati 65* showed the highest figure with regard to the number of layers of cells with their thickness in the sub-epidermal mechanical cylinder, the number of vascular bundles in the sub-epidermal and sub-cortical rings, the radial and the tangential diameters of vascular bundles in the sub-cortical rings, total number of vascular bundles in the “stem,” the number of air chambers in the sub-epidermal rings and the length and breadth of fibres, whereas with regard to other characters it may have the intermediate

TABLE 1. —THE DIMENSIONS OF MEASUREMENTS AND COUNTINGS OF DIFFERENT ANATOMICAL CHARACTERS OF THE STEM.

Vary.	Serial number of filices.	Diameter of the inter-nodes.	Number of layers of cells in sub-epidermal fibrous cylinder.	Thickness of cells in μ sub-epidermal fibrous cylinder with standard error.	Number of bundles in sub-epidermal ring.	Tangential diameter of bundles in sub-epidermal ring in μ with standard error.	Radial diameter of vascular bundles in sub-epidermal ring in μ with standard error.	Extra number of vascular bundles in sub-epidermal ring.	Number of vascular bundles in sub-epidermal ring.	Tangential diameter of vascular bundles in sub-epidermal ring in μ with standard error.	Radial diameter of vascular bundles in sub-epidermal ring in μ with standard error.	Extra number of vascular bundles in sub-epidermal ring.	Total number of vascular bundles in section.	Number of sub-cortical bundles.	Length of fibres in μ with standard error.	Breadth of fibres in μ with standard error.	Length of vessels in μ with standard error.	Breadth of vessels in μ with standard error.
<i>Juncea 34</i> ...	1	6.0	3	7.26	21	135.53	126.66	3	21	146.66	150.10	3	18	21	682.6	12.64	196.9	51.0
	2	5.5	3	7.86	23	129.10	142.73		23	119.10	162.73	4	50	23	838.1	15.27	206.7	51.0
	3	5.0	3	7.11	23	126.00	120.53		23	121.66	141.40	10	46	23	452.6	12.81	111.0	51.0
<i>Iberica</i> ...	1	6.0	2.3	6.13	25	110.00	131.06	8	25	125.53	217.50	6	56	25	638.8	14.17	116.1	51.3
	2	5.5	3.4	7.25	20	135.00	135.53	8	20	127.53	172.50	10	52	20	658.1	14.55	9.81	45.2
	3	5.5	3.1	6.30	24	104.00	108.53	3	24	110.53	119.40	6	27	24	689.4	17.5	9.51	28.5
<i>Banksiana 65</i> ...	1	9.0	5.6	10.21	32	156.66	108.86	3	32	186.06	185.26	3	61	32	618.4	11.18	177.6	17.5
	2	7.0	3.1	8.21	28	155.00	109.40		28	161.66	190.00	2	59	28	852.6	13.4	110.8	17.5
	3	6.5	3.5	7.86	21	131.06	100.00	3	24	142.73	161.66	3	53	24	651.3	13.4	111.8	19.5
<i>Lactuca</i> ...	1	6.5	3	6.90	26	127.73	130.86	3	26	135.00	163.86	4	59	26	661.3	12.57	147.3	26.7
	2	4.5	2.3	5.58	25	109.40	81.40		25	116.66	124.10	4	62	21	435.5	12.57	127.2	24.3
	3	5.0	2.3	6.07	27	117.20	101.06		26	107.73	145.38	9	62	26	467.1	18.4	127.7	46.2
<i>Potamo 23</i> ...	1	5.0	2.3	4.76	18	107.50	97.50	1	15	118.86	138.86	3	39	18	469.7	14.87	7.61	30.2
	2	5.0	2.3	4.11	12	127.50	135.53	5	15	125.53	137.73	3	38	22	808.5	14.5	12.51	35.9
	3	5.0	2.3	7.11	19	125.20	90.40		12	121.40	166.66	7	49	19	502.1	27.6	12.00	35.4
<i>Phaseolus</i> ...	1	6.5	3.5	8.57	26	117.20	107.20		26	101.66	168.86	3	52	26	352.6	17.4	139.0	30.7
	2	6.0	3.4	8.27	22	165.53	125.66		22	165.53	127.20	3	48	22	315.8	7.9	91.8	4.8
	3	5.5	3.5	7.29	21	151.40	98.33		24	133.00	114.66	—	48	21	1040.8	10.7	133.3	20.7

[To face page 6.]

or lowest figure when compared with other varieties. All the six varieties examined may be distinguished from one another on the basis of only one character from the statistical point of view as follows :

TABLE II.—SHOWING THE THICKNESS OF THE SUB-EPIDERMAL FIBROUS CYLINDER.

Varieties.						Mean with error.
<i>Jhanji 34</i>	...	...	...	...	...	5.19 ± 0.14
<i>Dharial</i>	...	...	...	...	...	4.66 ± 0.15
<i>Badkalamkati 65</i>	...	...	...	...	...	6.22 ± 0.31
<i>Latisail</i>	...	...	...	...	...	4.33 ± 0.15
<i>Patnai 23</i>	...	...	...	...	...	4.44 ± 0.25
<i>Bhasamanik</i>	...	...	...	...	...	5.69 ± 0.19

The mean with standard errors for each variety were calculated by taking the measurements of the first internode of the three tillers together, which has been shown in Table II. The difference between the means would be significant, when it is more than twice the standard error of difference.

The difference between *Jhanji 34* and the rest of the varieties has been significant, i.e. with regard to the thickness of the sub-epidermal mechanical ring *Jhanji 34* may be differentiated from *Dharial*, *Badkalamkati 65*, *Latisail*, *Patnai 23*, and *Bhasamanik*. Similarly *Dharial* may be distinguished from *Bhasamanik*, *Badkalamkati 65* from *Latisail* and *Patnai 23*; *Latisail* from *Bhasamanik* and *Patnai 23* from *Bhasamanik*.

Similar comparisons may be made by taking other anatomical characters under consideration.

Tilleral Variation.—There was no distinction between the main and other tillers in all the varieties so far as the following characters are concerned, e.g. the number of cells in sub-epidermal fibrous cylinder and their thickness, the number of vascular bundles and the number of air chambers in the sub-epidermal ring, the radial diameter of vascular bundles in sub-cortical ring. Only in case of *Patnai 23*, the thickness of the sub-epidermal fibrous cylinder in the main tiller was much less than that in other tillers (Table I). But distinctions between the main and other tillers were obtained in the majority of cases with regard to radial and tangential diameter of vascular bundles in the sub-epidermal ring, the number of vascular bundles and their tangential diameter in the sub-cortical ring (Table I). It is thus evident that in mechanical development all the tillers behave similarly, the distinction which has been noted in case of few characters might be attributed to local requirements. All the tillers in a plant are subject to the same environmental and cultural conditions. Hence the tillers by the similar development of the mechanical cells and tissues in them confirm the idea that all are of equal value to the plant and their mechanical system is not influenced by their length, age, and position in the plant.

Thus it may be concluded that the general principle of construction observed by the author in case of the different species in the genus *Oryza* (1941) remains the same even in the varieties of a species, viz. *O. sativa*. But there exist variations in dimensions and in number of different mechanical cells and tissues, which were quite remarkable.

SUMMARY.

Six varieties of transplanted paddy were examined. Samples were obtained from any three tillers of single plant of each variety.

Samples were examined both in section and maceration. Good results were obtained from safranin-haematoxylin stain with regard to sections and safranin in the case of macerated materials. Both sections and macerated materials could be preserved in glycerine alcohol solution (10 p.c. glycerine in 50 p.c. alcohol) for three months.

Measurements and countings of anatomical characters have been made from statistical point of view; the mean value with their standard errors for all the characters have been recorded.

Variation of different anatomical characters in the different varieties and different tillers of the same plant have been studied.

The general principle of construction observed in case of genus *Oryza* remains the same in varieties and tillers examined. But the variation in dimension and in number of different mechanical cells and tissues was very remarkable.

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DESCRIPTION OF PLATE.

- Fig. 1.—Transverse section of the first internode of the first tiller of *Badkalamkati 65* showing the free fibro-vascular bundles within the sub-epidermal ring.
- Fig. 2.—Transverse section of the first internode of the third tiller of *Jhanji 34* showing extra fibro-vascular bundles within the sub-cortical ring.
- Fig. 3.—Transverse section of the first internode of the first tiller of *Latisail* showing the extra number of fused and scattered fibro-vascular bundles in the sub-epidermal and scattered fibro-vascular bundles in the sub-cortical ring.
- Fig. 4.—Transverse section of the first internode of the third tiller of *Dharial* showing the embedded fibro-vascular bundles in the sub-epidermal ring.
- Fig. 5.—Fibres, vessels, and Parenchyma from the macerated materials of the first internode of the first tiller of *Patnai 23* in the low power.
- Fig. 6.—Fibres, vessels, and parenchyma of the above materials under high power.

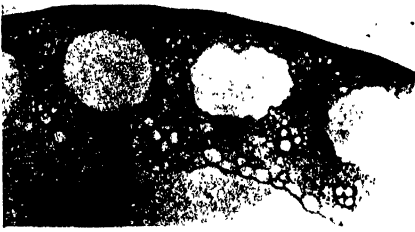


Fig. 1.



Fig. 2.

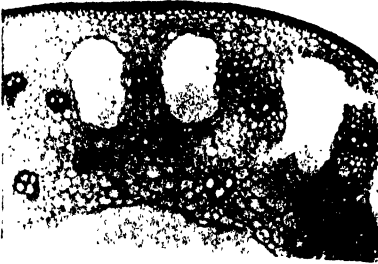


Fig. 3.



Fig. 4.



Fig. 5.

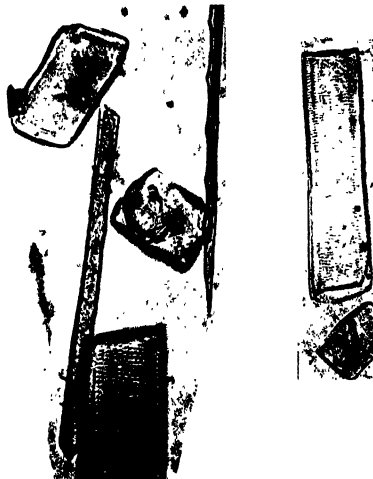


Fig. 6.

II.—A RAPID CATOPTRIC TECHNIQUE FOR RECOGNIZING AXIAL ILLUMINATION OF THE SUBSTAGE CONDENSER.

By A. G. OETTLÉ.

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THE adjustment of the substage condenser presents difficulties because substage illumination is the result of several independent manipulations of mirror and condenser. These manipulations are often carried out by trial and error until the illumination is judged satisfactory, but this empirical method is not adequate, especially for photomicrography, since photographic plates are more sensitive to uneven illumination than the human eye.

The technique which is adopted for adjusting the substage condenser must have three independent aims :

1. To illuminate the condenser along its principal axis.
2. To focus the condenser in the plane of the object.
3. To make the axis of the condenser coincide with that of the objective.

The present paper deals with the first of these three. In the past, axial illumination received relatively little attention, and writers on the use of the microscope seem to have been more concerned about centration of the substage condenser. Nevertheless axial illumination is quite as important. In the first place, the condenser is designed for illumination along the principal axis, and beams oblique to this axis produce aberrations. In addition, the microscope is an optical bench bearing a number of lens systems aligned in the optical axis, along which the illumination should obviously be directed.

Other Methods.

(a) FOR AXIAL ILLUMINATION OF THE MICROSCOPE.

The simplest method of illuminating the microscope along its axis is described by Beck (1921), but this method is not likely to be used very often. All lenses are removed. The eye is placed 6 or 8 inches above the tube and moved until the lower end of the microscope tube is central with the upper edge of the draw-tube. Then, without moving the eye, the mirror is moved until the light appears in the centre of these apertures.

Barnard (1918) described a similar technique in which the eyepiece is the only lens left in the microscope. Light is sent up the tube and axial illumination is recognized by the projection through the eyepiece of a complete circle on to the ceiling.

These two methods are convenient if the mirror, microscope, and lamp can be fixed in position, and alignment is obtained once and for all.

(b) FOR AXIAL ILLUMINATION OF THE CONDENSER.

Where the positions of the mirror, lamp, or microscope cannot be fixed permanently, other techniques for axial illumination are needed, which can be applied when the condenser is in place. The existing methods are all dioptric, and depend on the appearance of the image which the condenser forms in the object field. A low-power objective is usually necessary to examine the field, and the other adjustments of the condenser must be approximately correct. If the slide is under an oil-immersion objective these tests become very difficult to apply because of the smallness of the field.

If the condenser is already centred, the simplest way of obtaining axial illumination is to direct the light into the centre of the mirror and then to adjust this until the image of the light source or field diaphragm is in the centre of the object field. In the centred condenser the axis coincides with the centre of the field of the objective. When an image lies symmetrically about the optical axis of a lens, the rays which produce it must also be symmetrical about the axis.

Bright-ground Condensers.

I find an excellent indication of axial illumination is the appearance of the image which the condenser forms in the field of view. If the coloured haloes round this image are uniformly symmetrical or absent, the condenser is illuminated along its axis. This test has been described previously by Shillaber (1944) as "one of the quickest and simplest tests for the centration of the condenser." This statement is inexact. The test indicates axial illumination, for which the condenser has been designed and corrected, so that any chromatic aberration which is present will be symmetrical about the principal axis. With oblique light, new aberrations are introduced, e.g. coma and astigmatism, and these colour fringes are no longer symmetrical. The position of the condenser relative to the objective cannot affect the corrections of the condenser. Shillaber's interpretation of the test depends on his technique. He always directs the image of the light source into the centre of the field of view. Then, if the coloured haloes are deformed (indicating that oblique light has been used for projecting the image of the source into the field of the objective), the condenser must be eccentric. If the colour fringes are uniform, i.e. if the illumination is axial, the condenser must be centred. In this sense only can the test become a test of alignment.

Dark-ground Condensers.

Three methods are given for this type. Belling (1930) suggests that axial illumination be obtained for a bright-ground condenser first and the dark-ground condenser be inserted without moving the lamp or mirror. Gage (1934) advises that the mirror be adjusted "until the smallest, brightest spot is in the middle of the condenser face." Shillaber (1944) recommends that the condenser be put out of focus and the mirror moved until the hollow cone of light forms a perfect annulus on the slide.

New Method.

This method is rapid, and may be applied to any type of condenser and is independent of the centration or focus. It does not involve an examination of the object field, so axial illumination may be regained rapidly if the mirror is accidentally displaced, even when the slide is under an oil-immersion objective.

PROCEDURE.

1. Place the blue filter usually supplied with the microscope in the substage ring below the condenser, with the polished surface towards the source of light.

2. Direct the beam of light from the lamp on to the substage mirror. (If the substage mirror is not being used, then the light is sent on to the filter directly and the angle of incidence of the light is altered by moving the microscope or the source.)

3. Adjust the mirror so that it reflects the light on to the under-surface of the blue filter. When the mirror is in an approximately correct position, a dim spot of light may be seen near the lamp. This spot is a reflection from the under-surface of the filter and should be roughly circular in shape. If the lamp does not completely illuminate the filter, the circular outline of the dim reflection will be deformed. Then the direction of the beam of light should be altered by moving lamp or mirror until the whole of the filter is illuminated, resulting in a circular outline in the reflection.

4. Adjust the mirror until the dim spot of light is superimposed on the aperture of the field (lamp) diaphragm. Narrowing the field diaphragm will make this easier.

When this has been done, the incident light will strike the blue filter at right angles to its surface and the light will be directed along the axis of the substage. The mirror should not be moved in any subsequent procedure. The blue filter may now be removed.

OCCASIONS IN WHICH THE FILTER NEED NOT BE INSERTED.

1. Reflecting dark-ground condensers have a plane undersurface from which the reflection can be obtained directly.

2. In certain cases a —3 dioptré correcting lens is used in the substage ring, e.g. when using the Zeiss achromatic condenser N.A. 1.4. Here the correcting lens will produce a reflection which may be used in the same way. This correcting lens has a concave surface, so the diameter of the reflection will vary with distance from the microscope.

3. The Zeiss aplanatic condenser N.A. 1.4 also produces a reflection by itself, although the front surface is convex. Axial rays pass through the front lens and strike the second component normal to its front surface. The small proportion which is reflected here retraces its path back through the front lens, to form a spot of light with coloured fringes near the light source.

COMMENT.

The amount of light which is reflected is always small, so that it may be necessary to increase the visibility of this if the light source is not intense or if it is glaring. In the first case, the room should be darkened. If need be, a mirror cut to fit the substage ring may be used instead of the blue glass filter, to give a very bright reflection. This is not likely to be necessary where a powerful lamp is used. A ground-glass diffusing plate used at the lamp diaphragm increases the glare and should be removed while the reflection is studied.

The method assumes that the plane of the substage filter holder is parallel to that of the condenser sleeve. This may be verified by comparing the reflection from a plane surface held in the condenser sleeve (e.g. the undersurface of a reflecting dark-ground condenser) with that from the blue glass filter. If the filter is parallel to the condenser sleeve, the two reflections will be superimposed. If these reflections do not coincide, the filter holder is out of alignment. One may correct this mechanically or one may allow for the existing error and return the reflection from the blue filter to the position which was found empirically to indicate axial illumination of the cardioid condenser.

DISCUSSION.

The principle involved is elementary. If light strikes a plane glass surface about 5 p.c. is reflected at normal incidence. When the reflected beam is made to coincide with the incident beam, i.e. when the angle of incidence becomes zero, the light is normal to the surface. Since this surface has been shown to be at right angles to the axis of the condenser the light will be directed along this axis.

This technique deserves wider adoption. It is used in physics laboratories, but seems to have been overlooked by microscopists. It has been described for the cardioid condenser by Gage (1920), Kolmer and Boerner (1938), and the present author (1946), but even for this condenser the technique is rarely applied. Gage thought that the reflection is formed at the upper surface of this condenser, while Kolmer and Boerner, without producing any evidence, claimed that it arises from the lower surface. The reflection, as already indicated, undoubtedly arises from the lower surface of this condenser. This is evident from the following considerations :

1. When parallel light is used, the reflection has the same size and shape as the under-surface. The cardioid condenser produces a circle of light, while the paraboloid condenser, with its central stop in the under-surface, forms an annular reflection.
2. Homogeneous immersion of the upper surface of the condenser does not affect the reflection. It cannot therefore arise from the upper surface. There are no other plane surfaces.

The success of this technique is independent of the centration and focus of the condenser and of the centration of the mirror. Consequently, no attention need be directed to the problem of aligning the mirror, as recommended by Shillaber (1949), provided the whole of the filter is illuminated. The position of the lamp is not of great importance, as long as its rays fall on the substage

mirror. Once the position of the mirror is correct the lamp and microscope will be accurately aligned as if they were held on an optical bench.

SUMMARY.

A technique is described for illuminating the condenser along its axis. A plane glass disc is placed in the filter holder of the substage. When the reflection formed by this disc is superimposed on the light source, axial illumination is achieved.

ACKNOWLEDGMENTS.

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III.—THE DESPECIALIZATION OF NEOPLASTIC CELLS.

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THE fundamental attribute of neoplastic cells is that they are not completely subordinated to the control of the organisms in which they occur (Reimann, 1938 ; Ewing, 1929). With the exception of true terata, neoplasms are composed of anaplastic cells, that is, cells which are incapable of complete specialization (Ewing, 1928), and may therefore be termed despecialized. Since the adaptation of a cell to the performance of a special role in the economy of an organism (cellular specialization) is dependent on the correlated changes within the cell itself (cellular differentiation), persistent despecialization can result from the loss of specific qualities by a cell (dedifferentiation) insofar as this loss is compatible with continued viability.

Neoplasms can be graded according to the degree of despecialization which they show and malignant cells, since they represent the culmination of the neoplastic sequence, provide the most suitable example of anaplasia for consideration. The main features of malignant cells include continual proliferation, autonomous growth, invasion and infiltration, metastasis, and dedifferentiation. These properties are primary to the operative pathological effects of malignant tumours and are causally interconnected. For example, differentiation and the rate of cell division are inversely correlated in both normal and malignant cells (Berrill, 1935, 1943), and irreversibly dedifferentiated proliferating cells are *ipso facto* autonomous inasmuch as the organization of a metazoan depends on the co-ordination of specialized and differentiated cells. Continual division, autonomous growth and, in some instances, morphological plasticity associated with dedifferentiation, result in invasion and infiltration of adjacent tissues which cause metastases in turn. As has been concluded elsewhere (Reimann, 1938 ; Schrek, 1936), cellular dedifferentiation is the fundamental characteristic of malignant cells at the cellular level of organization. It appears that the basic causes of neoplastic growth should be sought in factors which bring about inherited and persistent failures of cellular differentiation.

During development the potencies of somatic cells are progressively restricted, and in adult mammals definitive histological-type differentiations are normally irreversible and inherited by the cells of a given lineage. The site of cellular differentiation is almost exclusively cytoplasmic and nuclear changes are relatively insignificant (Harrison, 1940 ; Jennings, 1940). Since there is no clear evidence of regulated and constant differences between the genotypes of the

somatic cells of an individual animal, it has been concluded (Wright, 1941) that cellular differentiation is inherited through the cytoplasm.

A synthesis of existing knowledge of cytoplasmic inheritance, plasmagenes, and nuclear inductors, has led to the view (Wright, 1945) that cellular differentiation is achieved by the differential utilization of nuclear gene products by the cytoplasm of the various kinds of specialized cells in an organism. The pattern of cell processes is inherited and finally becomes fixed in the definitive types of cells. The specific products of nuclear genes have hapten-like properties and form the prosthetic groups of specific cytoplasmic proteins and, while nuclear genes determine the availability of these specific products, the cytoplasm regulates their disposal in accordance with its inherited constitution.

Some at least of the specific gene products are necessary for the special functions of a given differentiated cell. If such a product is inessential for the viability and reproduction of the cell, it follows that its removal or dysfunction could cause dedifferentiation and despecialization. The extent of the consequent degeneration will vary with the importance of the particular nuclear inductor in the normal cellular processes. The functional loss of the gene product might be due to a modification of the nuclear gene concerned or of a plasmagene directly utilizing or indirectly modifying the product, or to other agencies such as viruses. Differences of detail between tumors of the same grade, even if derived from the same kind of parent tissue, would be expected if different nuclear inductors were involved. Specific substances other than products of nuclear genes but necessary for full differentiation might play a similar role in despecialization.

On the other hand, the majority of malignant growths—to take a particular kind of neoplasia—show a characteristic complex of properties. It is possible that in such a group of tumors a common basic factor is involved. Irrespective of particular differentiations, the normal functioning of cellular processes depends on the integral organization of living protoplasm. The modern conception of protoplasmic structure (Bensley, 1943) is that at least three distinct and known components are involved—protein microfibrils forming a structural framework; a system of submicroscopic particulates; and a continuous aqueous phase. The morphological and, to some extent, the chemical organization of protoplasm depends on the co-ordination of the structural proteins. Disorganization of protoplasmic structure and especially inco-ordination of the protein framework would lead to dedifferentiation.

Fibrous structural proteins participate in the sol-gel transformations of protoplasm (Bensley, 1943) and, consequently, in nuclear and cell division, which depend on such changes (Chambers, 1917, 1924; Lewis, 1942). As the mitotic spindle is birefringent (Schmidt, 1936) and a viscous gel (Chambers, 1917), it is almost certainly composed of elongated protein microfibrils. Inco-ordination of protein microfibrils could therefore result in aberrations of division processes and of the spindle and chromosomes. It could also account for the observed changes in the responses of tumor cells to ultra-centrifugation (Cowdry and Paletta, 1941; Guyer and Claus, 1935, 1939, 1942), and alterations in water content (Auler, 1936; Okuneff and Nasarbekowa, 1934), and for the observed differences in the micromorphology of their protoplasm (Lewis, 1939, 1942;

Ludford, 1934). Since respiration is decreased and glycolysis increased by protoplasmic disorganization (Fleischmann, 1939 ; Korr, 1939), the characteristic metabolism of tumor cells is also explicable on the same basis. It is possible that thiol groups are exposed by the inco-ordination of the fibrous units and are partly responsible for the changes in metabolism as they tend to elevate glycolysis (Lipmann, 1942) and inhibit or stimulate certain enzyme processes.

The suggested mechanisms of despecialization and their relations to various aspects of tumor growth will be discussed in fuller detail elsewhere.

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IV.—NOTE ON THE TAXONOMY OF *SPIRILLINA LIMBATA* VAR. *DENTICULATA* BRADY.

By C. D. OVEY, B.Sc., F.G.S. [Dept. of Zoology, British Museum
(Natural History)].

WHILE working on the material collected on the British, Australian, and New Zealand Antarctic Research Expedition, 1929–30, W. J. Parr has encountered certain specimens of *Spirillina* which were similar to those described and figured by Chapman (1907, p. 133, pl. X, figs. 6a–c) under the new specific name *Spirillina denticulo-granulata* and which the latter obtained from littoral gatherings on the shores of Victoria, Australia. In a recent letter W. J. Parr says, “Brady, in the *Challenger* Report, described a form under the name of *Spirillina limbata* var. *denticulata* (1884, p. 632, pl. LXXXV, fig. 17). While *S. limbata* Brady is a symmetrical form with the tube gradually increasing in diameter, the figure of the [variety] *denticulata* shows a form in which the centre is the highest point. In my experience, this is formed only in assymmetrical *Spirillina* and I therefore suspect that the denticulate ornament is confined to the figured [dorsal] side and that the other side is flat and probably beaded. I am led to this conclusion as while, according to Nuttall [1927, p. 234], Brady’s figured specimen came from Bass Strait, all of the examples of *Spirillina* showing a surface like Brady’s figure that I have seen from this area, have the other side as I have described. I feel sure that Brady has the same form as that later described by Chapman [*op. cit.*] as *S. denticulo-granulata*.”

In order to satisfy Parr’s doubts the present writer at the former’s request turned over Brady’s figured specimen,* and discovered that the test was in fact assymmetrical. The unfigured side is slightly concave, curving gently in towards the centre becoming flat and disc-like over a short distance near the centre, while the whole surface is covered with small beads apart from the last formed whorl which is quite smooth.

Besides the figured specimen, Brady has mounted in the same cell five other syntype specimens, three of which are young. These three show the beading much more distinctly than the adults, but in each case the outer, last formed whorl is free from beads. These syntypes of Brady match exactly Chapman’s figures (*op. cit.*) and therefore *Spirillina denticulo-granulata* is without doubt a synonym of *S. limbata* var. *denticulata* Brady; but evidently Chapman had not had the opportunity to examine Brady’s original material. In the former’s description of the “superior [dorsal] face” (that side which Brady figured), Chapman recognized, as he says (*op. cit.*, p. 134), that the “surface is ornamented with a series of depressions or short grooves at right angles to the border, as in *S. limbata* var. *denticulata* Brady” (and here he gives reference to Brady’s figure). He then goes on to describe the “interior surface” as concave from

* Brady’s *Challenger* types are preserved in the collections of the Department of Zoology at the British Museum (Natural History).

the inner border to the last whorl, while the surface is almost entirely covered with granulations ; but there is no further reference to Brady. Even Chapman, therefore, recognized the similarity of the dorsal side of his species and Brady's variety, but he did not know of the beaded granulations on the ventral side of the figured specimen.

It is a curious thing that Brady himself never noticed this fact, which is evident from his description, especially as he mounted six syntypes, including the figured specimen, some showing one side and some the other (a seventh small specimen is also mounted on the same slide but this appears to be a young *S. viripara* Ehr.).

The present writer considers Brady's variety to be closely related to *Spirillina wrightii* Heron-Allen and Earland (1930, p. 181, pl. IV, figs. 54-58), to *S. dimidiata* Wiesner (1931, p. 128, p. XXI, fig. 250) and to *S. limbata* var. *papillosa* Cushman (1915, p. 6, pl. II, fig. 4) but not to *S. limbata* Brady (1884, p. 632, pl. LXXXV, figs. 18-21). The three former and Brady's variety have the common character of beaded granulations on the ventral side, raised carinate or limbate borders to the coil on the dorsal side and all are slightly convex on this side and flat or concave on the ventral. *S. limbata* Brady on the other hand is, to use Brady's own words, "planospiral, thin, equilateral, discoidal," and although the coil borders are raised on both sides, the whole is much more closely coiled. *S. limbata* var. *denticulata* Brady and var. *papillosa* Cushman should, in the writer's opinion, therefore be brought to specific status respectively, thus—*S. denticulata* Brady and *S. papillosa* Cushman.

SUMMARY.

(1) *Spirillina denticulo-granulata* Chapman is a synonym of *S. denticulata* Brady (= *S. limbata* var. *denticulata* Brady).

(2) *S. limbata* Brady is not closely related to the so-called varieties (var. *denticulata* Brady and var. *papillosa* Cushman) being a symmetrical, biconcave, closely coiled form with neither granulations on the one side nor grooves at right-angles to the periphery of the whorls on the other.

(3) The species *S. denticulata*, *S. dimidiata*, *S. papillosa* and *S. wrightii* belong to a group of closely allied forms all of which are assymmetrical with the dorsal side slightly convex, and with granulations on the slightly concave or flat ventral surface. At least the outer half of the tube in the last formed whorl on the ventral side is free of granulations.

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V.—A MASS-PRODUCTION METHOD FOR PARAFFIN AND FROZEN SECTIONS.

By ROBERT H. WATTS, Technical Assistant, Department of Physiology,
King's College Medical School, Newcastle-upon-Tyne.

INTRODUCTION.

THE techniques and staining methods described in the histological literature almost invariably deal with the preparation of single specimens. In many teaching establishments large numbers of slides are necessary and mass production methods essential. The following procedures for paraffin and frozen sections are employed in this department where some hundred batches of two hundred slides are prepared each year. No originality is claimed for specific details of technique and apparatus.

METHOD FOR PARAFFIN SECTIONS.

- (1) Ribbon sections are cut from the paraffin block in the usual way, and placed in rows upon a sheet of paper.
- (2) Single sections are cut from the ribbon with a scalpel and tapped on to the surface of a jar of warm water. The water should be warm enough to flatten the sections in about half a minute. The vessel should be sufficiently narrow to avoid rapid surface cooling, or if a wide vessel is used, thermostatically controlled.
- (3) In order that large numbers may be stained simultaneously, the sections are floated on to cover-slips. This is effected by dipping the albumenised cover-slip beneath the water at right-angles to the surface, touching the floating section with the face of the cover-slip and lifting it clear of the water. The section should adhere centrally to the cover-slip. Circular slips of No. 2 thickness and $\frac{7}{8}$ -inch. diameter are normally employed.
- (4) The cover-slips are now placed in wooden racks and left to dry in an oven controlled at 38° C. overnight. Each drying rack is designed to hold sixty cover-slips, and is made from a block of wood approximately $9 \times 5 \times 1$ inches, with twelve saw cuts across its face in which the cover-slips rest. The cuts are sloped slightly to give the cover-slips a backward tilt.
- (5) From the drying racks the cover-slips are transferred to staining racks constructed from three 3-inch lengths of No. 0 B.A. threaded brass rod, which are set three-quarters of an inch apart and bolted or soldered to end plates. Each rack has an arched wire handle and is compact enough to fit into a vessel 5 inches wide. This rack also is capable of holding sixty cover-slips.

- (6) Earthenware vessels fitted with lids, such as casserole or butter-dishes, are used to contain the reagents for de-waxing, hydrating, staining, etc., and for convenience these are best arranged in sequence on shelves, with the de-waxing vessel on the uppermost shelf. Procedure through the vessels is essentially as for single sections.

Where regressive staining is required, differentiation may be controlled by removing a single cover-slip from the rack with forceps, placing it face downwards on a clean slide (to prevent drying) and viewing through the microscope. This slip may be returned to the rack.

Triple staining techniques, such as that of Mallory, can be used satisfactorily.

- (7) To mount the sections, clean slides are laid in rows upon a tray large enough to hold one hundred slides. A drop of balsam is placed in the centre of each slide in one row (about twenty at a time), and the cover-slips with the sections face downwards are carefully manœuvred into position with a pair of forceps. On account of the balsam drying, it is important not to mount more than one row of slides at a time, and to keep the rack of cover-slips immersed beneath the clearing agent while mounting is proceeding.

Note.—If comparative staining is required, two slips stained by different methods may be mounted on the same slide.

STAINING METHOD FOR FROZEN SECTIONS.

- (1) A batch of about twenty sections is taken from water or from storage fluid, and passed through all necessary reagents in a rectangular staining box made from brass wire gauze, and measuring $3 \times 1\frac{1}{2} \times \frac{1}{2}$ inches. The box has a close-fitting lid and a vertical stiff wire handle. The mesh of the gauze is sufficiently fine to retain the sections, but wide enough to permit the easy passage of reagents.
- (2) In the reagents the box should be gently agitated from time to time to avoid clumping of the sections, and at each change drained of surplus fluid on a wad of blotting paper.
- (3) The sections are floated individually on to slides from the absolute alcohol. They are cleared *in situ* and mounted as for single sections.

My thanks are due to Mr. A. E. Needham for his kind assistance in connection with this paper.

VI.—THE STRUCTURE OF THE SKIN AND LEATHER MANUFACTURE.

By MARY DEMPSEY.

(Paper read before the Industrial Section, February, 25th, 1948.)

TEN PLATES.

IN making leather the tanner has two main objects in view :—

- (1) To render the raw skin imputrescible by applying a suitable tanning agent such as the vegetable tans, basic chromium sulphate, or oil.
- (2) To produce leather with suitable physical properties for particular purposes.

The most commonly used heavy skins ("hides") are from cattle and the most common light skins ("skins") are from calf, sheep, and goat.

From ox hide, leathers of widely different physical properties are produced. Sole leather, for example, for use in the English climate is firm, not very flexible, abrasion resistant, impermeable to liquid water but, what is so highly important for foot comfort and health, permeable to water vapour. At the other extreme, picker band leather must be very flexible and strong. A picker band is a narrow strap attached to the picker (a hammer) which by repeated rapid blows sends the shuttle backwards and forwards in the loom in weaving, so it has to withstand constant sudden sharp flexing and pulling. Between these in properties come machine belting leather which must be flexible and strong with little stretch.

Among light leathers sheep skins, for example, are made into leathers with a wide range of physical properties. Roller leathers are comparatively firm, dense leathers; the leather is bedded down on to small rollers used in cotton spinning to pick up the yarn and hold a twist in it as they pass it through. The grain of the leather must therefore be smooth, resilient, and resistant to constant fine abrasion. Sheep skin clothing leathers such as are used for leather jerkins must be flexible, able to stand machine stitching, and capable of being sharply turned at seams. Gloving leathers must be extremely flexible and possess the special characteristic known in the Industry as "run." This is a plastic property by which if the leather is pulled it will go long and narrow, and stay like that; but it can easily be pulled back again. A high quality leather glove should "give" to the shape of the hand and allow freedom to the fingers: at the same time it should have enough resilience that it does not "bag" around the joints, but not be so elastic that it grips the hand and feels tight.

Leather is made mainly of interweaving collagen fibres (or fibre bundles), and the physical properties of the leather are the sum of the physical properties

of the fibres and their manner of interweaving. The condition of the collagen fibres and their interweaving constitute what we call, in a narrow sense, the "fibre structure" of the leather. Since some of the physical properties of the fibres are visible or may be inferred from the appearance of the fibre structure it is possible by means of the microscope to assess the quality of a given leather and its suitability for a given purpose. It is also often possible to see the reason for the failure of a faulty leather.

We are not, however, only interested in the finished product: it is necessary in the complicated processes of leather manufacture to examine the fibre structure throughout manufacture, as this is, up to the present, often our only way of knowing the immediate precise effect of a process upon the hide or skin.

In addition, it is obviously important for the tanner to know as much as possible about his raw material, and the histology (as distinct from the "fibre structure") of the various skins he uses must be studied. I will therefore start by illustrating the histology of leather-making skins, then the method of making microscopical assessments of the appearance of the fibre structure and finally show the typical micro-structure of various leathers and the diagnosis, by means of the microscope, of faults in leather. A considerable amount of the original work (with special reference to fibre structure) is due to Kaye (1929) and Marriott (Jordan Lloyd and Marriott, 1932). It is not possible in this communication to give full references; a full list is given in "Progress in Leather Science, 1920-1945."

Figs. 1-5 show the general structure in cross-sections of ox hide and goat and sheep skins. The basic structure is the same in each. The relatively thin epidermis covers the *pars papillaris* (the "grain layer") through which run the hairs and related structures (see fig. 6). The grain layer merges into the *corium major* (the "corium") which consists mainly of bundles of collagen fibres interweaving in all directions. The relative depth of the grain layer to the full thickness is small in the ox, very much greater in the goat, and still greater in the sheep. The appearance of fibre structure of a sample is mostly assessed in the middle of the corium.

Figs. 3-5 show how sheep skin varies at different sampling positions. The depth of the grain layer is almost constant but the corium varies considerably. Similar variations occur in heavy hides and the difference in these in fibre structure will be illustrated later (see fig. 22) in sole leather. A large amount of triglyceride fat is stored in sheep skins, mainly at the junction of the grain and corium: this amount can also be seen to vary according to sampling position. Fat is less readily stored in the skin by cattle but some ox hides show a considerable amount in depots of fat cells scattered through the corium mainly over the kidney region (O'Flaherty and Roddy, 1934, 1935). Fig. 6 shows the structure of the grain layer in greater detail.

The tanner removes the epidermis and hairs, and the basement membrane (fig. 7) between the epidermis and true skin forms the grain surface of the leather. The nature of this membrane is in some dispute. Küntzel (1925), for example, considers it is collagen, but we have concluded that since it can be impregnated with silver and can be seen in tangential sections to consist of a network of branching anastomosing fibrils, it is reticulin.

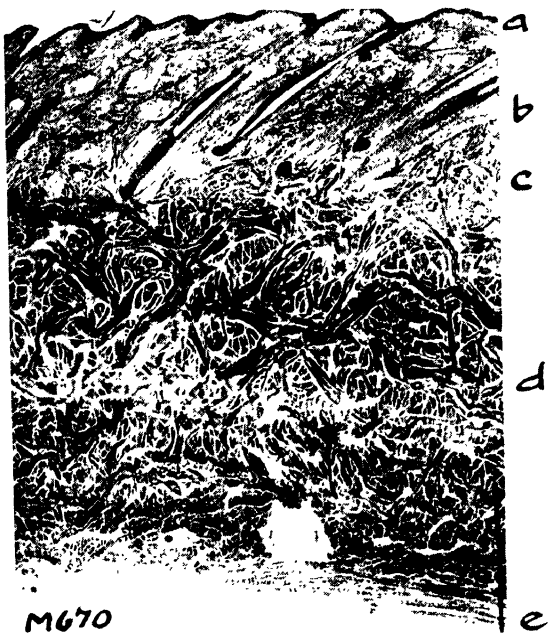


Fig. 1.



Fig. 2.

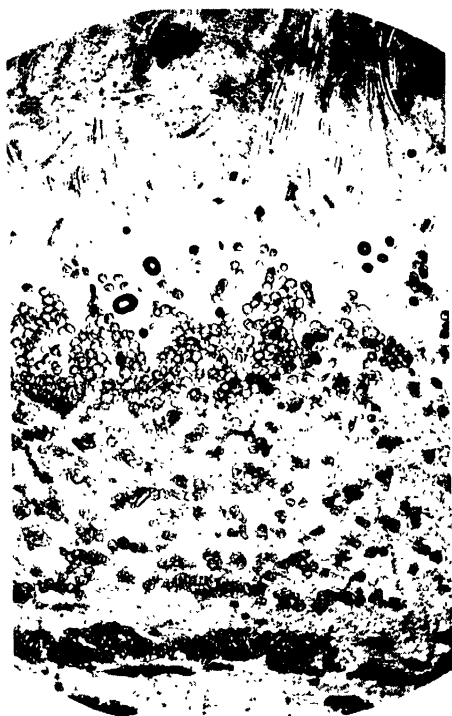


Fig. 3.



Fig. 4.



Fig. 5.

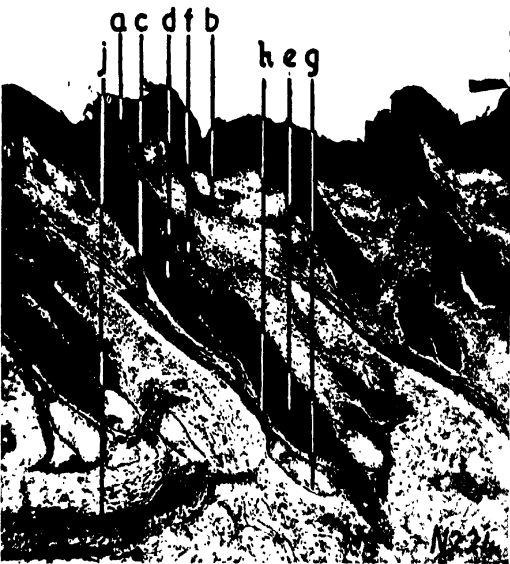


Fig. 6.



Fig. 7.



Fig. 8.

[To face Plate III.]



Fig. 9.



Fig. 10.



Fig. 11.



Fig. 12.

[To face Plate II.]



Figs. 13-15.

[To face p. 23.]

Elastin fibres form a considerable proportion of the connective tissue in the grain layer (fig. 8) and seem to have a special strengthening and supporting function. Figs. 9 and 10 show elastin fibres attaching the *erector pili* muscle at one end to the hair follicle wall and at the other to the surface of the dermis.

The various structures and fibres of the grain layer are important because of their influence on the smoothness and natural contours of the grain which are prized features, especially of light leathers.

In the corium the morphology of the collagen fibres and fibre bundles is of prime interest to the tanner. The amount of longitudinal splitting up into smaller fibres and fibrils shown at each stage in manufacture is intimately related to the quality of the finished leather. In addition the manner in which the fibres and fibre bundles are split is highly important, that is to say, whether the constituent fibrils remain well held together or are falling apart and separated.

Fig. 11 shows the cross-sections of fibre bundles in skin. During paraffin embedding the collagen fibres have shrunk and the encircling fibres can now be seen. It is well known (see for example Maximow and Bloom, 1942) that collagen fibres when plumped in dilute acid show, at intervals, constricting rings—the rings of Henle (fig. 12). Various explanations of the formation of these rings have been put forward, probably each of them true in part. We have evidence that at least sometimes they are formed in the skin by reticular tissue. Processes of manufacture must sufficiently loosen the reticular tissue so that the constituent fibrils lie minutely apart from each other although still held together in bundles as units, i.e., the fibre bundles must be well “split up”: but the reticular tissue must not be so destroyed that the fibrils fall apart from each other and the unity of the bundles lost—i.e., the fibre bundles must not be “separated.”

Figs. 13–15 illustrate splitting-up in limed ox-hide. Lime liquors must not only unhair the hides or skins, they must suitably open up the fibre structure so that the subsequent processes of tanning may proceed smoothly. Striations extending along the fibre bundles and detail in their cross-sections indicate the amount of splitting-up. Fig. 13 shows the most splitting-up of the three examples, and fig. 15 the least. In all three the longitudinal and cross-section outlines are sharp and clear which indicates that the fibre bundles are still well held together as units.

Figs. 16–18 illustrate separation. Separation is recognized by a diminution in crispness of outline of the fibre bundles, by a splaying-out of the cut ends of bundles and ultimately by a general loss of delineation and order in the weave pattern. These three photomicrographs show a considerable amount of fine splitting, but the clear delineation in fig. 16 shows that there is little separation: there is more in fig. 17 and a considerable amount in fig. 18. Fibre bundles which lack splitting-up will, of course, show a minimum of separation. Separation is generally a fault indicating loss of hide substance either owing to bacterial action or to chemical attack.

Collagen fibres take up water in acid and in alkaline solutions and they increase considerably in diameter and become a little shorter. Goods pass through alkaline lime liquors and subsequently acid tan liquors. Hence the turgidity of the fibres is another feature that is assessed microscopically.

Figs. 19–21 illustrate turgidity in limed pelt. The thickness of outline of an isolated collagen fibre (which may be regarded as cylindrical) will depend partly on the size of the fibre and largely on the difference in refractive index (R.I.) between it and the mounting medium. An unplumped fibre mounted in an aqueous medium will show thicker outlines than the same fibre plumped-up so that its R.I. is lowered towards that of the medium. Within the hide, as the fibres plump they become closely pressed against each other so that their outlines become thin and may even disappear. In this way detail may be lost and the fibre bundles look translucent and glassy. Fig. 19 is very turgid for limed pelt. Some of the thicker outlines in the photomicrograph are due to the thickness of the section itself, but all the outlines are crisp and sharp, the bundles appear glassy in contrast to fig. 20 and still more to fig. 21. Acid plumping is more vigorous than alkaline and if the hides are allowed to become over-acid plumped in the early tan liquors the whole fibre structure becomes quite distorted.

The final feature to be illustrated (there are others which time will not allow) is the angle of weave. The collagen fibres follow a wavy course through the leather and the slopes of the waves make an angle with the surface—the angle of weave. Figs. 14 and 15 show a high angle of weave; fig. 21 shows a low angle of weave. This feature is important because the longitudinal and transverse properties of the collagen fibres differ (Jordan Lloyd, 1943). Leather fibres abrade more easily longitudinally than transversely so that for sole leathers which are expected to be hard-wearing most of the fibre bundles should be “vertical” i.e., the slopes of the waves should point almost upright towards the grain. On the other hand the tensile strength of fibres is greatest along them, so that for belting leathers the “lower” the angle of weave (the shallower the waves), other things being equal, the stronger the leather.

Fig. 22 shows at (a) a typical high-quality sole leather with large fibre bundles, compactly woven together at a high angle, taken from the best sampling position in the hide, and at (b), (c), and (d) the inferior quality obtained at other sampling positions.

Fig. 23 shows a typical high-quality belting leather with a medium angle of weave and a large amount of splitting-up.

Fig. 24 shows a picker band with medium angle of weave and a very large amount of fine splitting-up without undue separation.

Fig. 25 shows a high-quality gloving leather. The very fine splitting-up shown together with the modicum of separation is related to the “run” of the leather.

The same kinds of co-relations have been found for other types of leather.

FAULTS IN LEATHER.

Faults may be due (1) to the condition of the living animal, (2) to damage caused during processing, or (3) to ill-usage. I will mention a few examples.

(1) Triglyceride fat stores in the skin will, if not removed, cause a variety of troubles during manufacture of light leathers, and will make the finished leather, both light and heavy, show unsightly grease stains. Fat in the leather



Figs. 16-18,

[To face p. 24.]



Figs. 19-21.

[To face Plate VII.]

A.

B.



C.

D.

Fig. 22.



Fig. 23.

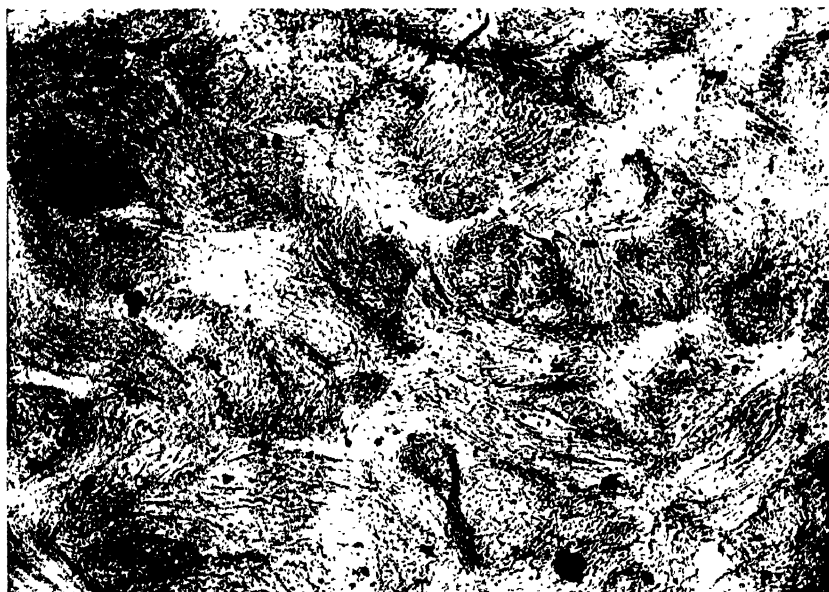


Fig. 24.

[To face Plate IX.]



Fig. 25.



(a)



(b)

Fig. 26.

[To face Plate VIII.]

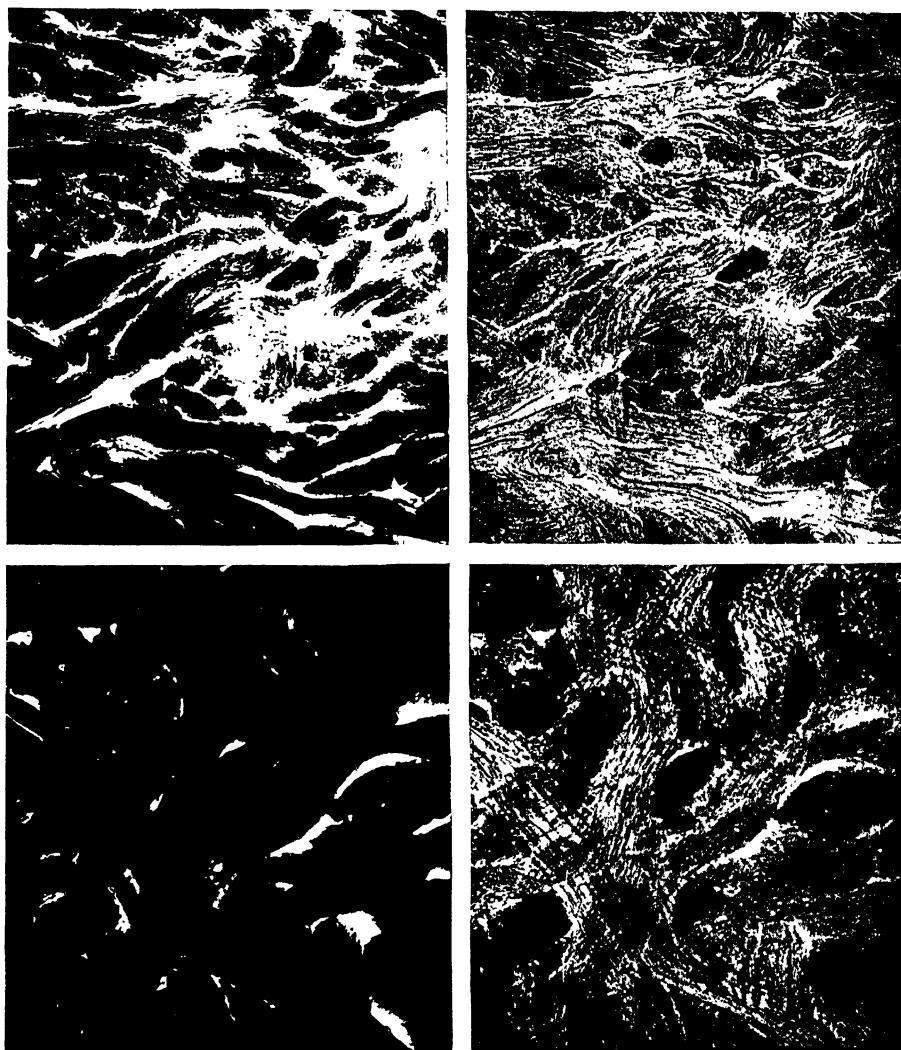


Fig. 27.

can be recognized by means of the ordinary histological staining technique with Scarlet R., but since some grease is added to the leather in the finishing processes, remnants of fat envelopes among the fibres must be looked for as confirmation of its presence in the living animal.

Scar tissue and other abnormalities due to diseases in the skin can also be recognized microscopically.

(2) There are various stages at which bacteria are likely to attack the goods—the most usual being during curing and soaking of the hides or skins. Bacteria cause a breakdown of the skin structures, apparent as over-opening up (excessive splitting-up and separation). Chemical damage during liming can also cause a similar appearance. The two are usually distinguished in the finished leather by stripping the tan out of the sections and then staining for bacteria (Raymond, 1937). Fibres which have been excessively opened up may, at later stages of manufacture, become re-stuck, so we must be able to recognize this re-sticking. We do so from the crinkling of the fibres, the presence of discontinuous longitudinal lines along the fibres, brittle-looking breaks in the sections and a glassiness often shown at the broken edges. A very tell-tale part of the leather for this kind of damage is the junction of the grain and corium.

When leather has been excessively opened up it may happen that the dye (where one is used) penetrates too freely among the fibres so that sections of the leather are opaque to visible light. Fig. 27 shows the detail revealed by the use of I.R. photomicrography (Curtis and Dempsey, unpublished, in preparation).

(3) One of the commonest ways of ill-using a leather is to dry wet shoes in front of the fire. Vegetable tanned leather will not stand heat while damp. The fibres become glued together, detail is lost and the weave becomes distorted and compacted (Robertson, unpublished). Fig. 26 shows a normal sole leather and an adjacent piece which has been dried before an electric fire. Chrome tanned leather (used for shoe uppers) is more resistant.

If soles wear out too quickly the microscope will reveal whether the sole was cut from an inferior part of the hide (see fig. 22) or from leather made from tainted hide, or whether the leather had been damaged by over-heating while wet.

In this account of microscopy in leather science I have not attempted to cover the whole field in any detail, but have surveyed the use of the microscope in fundamental histological work, and as a tool in controlling leather processes, judging quality in leather and explaining such faults as from time to time occur.

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DESCRIPTION OF PLATES.

- Fig. 1.—Ox hide, $\times 15$: (a) epidermis; (b) papillary layer (= grain layer); (c) junction of grain layer and corium; (d) *corium major* (= corium); (e) "flesh" layers of the corium. Del Rio Hortega's silver method.
- Fig. 2.—Goat skin, $\times 20$. The grain layer is relatively deep: the fibres of the corium are small and compactly interwoven. Note the subcutaneous fat cells. Scarlet R.
- Figs. 3-5.—Sheep skin at brich area, mid-flank and belly edge of shoulder, $\times 20$. The grain layer is deep and constant over the skin. The corium consists of finer fibres than in goat skin, and less compactly interwoven: it varies in thickness. Note the distribution of fat stores. Scarlet R.
- Fig. 6.—Grain layer of calf skin, $\times 55$: (a) epidermis; (b) blood capillary; (c) *erector pili* muscle; (d) sebaceous gland; (e) root sheath; (f) hair shaft; (g) sweat gland; (h) duct of sweat gland; (j) blood vessel. Heidenhain's hæmatoxylin.
- Fig. 7.—The fibrous basement membrane between the epidermis and the dermis, $\times 600$. Foot's silver method.
- Fig. 8.—Elastin fibres in the grain layer of calf skin, $\times 86$. Weigert's elastin stain.
- Fig. 9.—An *erector pili* muscle in ox hide, anchored near the surface of the dermis by elastin fibres, $\times 120$. Weigert's elastin stain.
- Fig. 10.—An *erector pili* muscle in ox hide anchored to a bulge in the side of the hair follicle by elastin fibres, $\times 120$. Weigert's elastin stain.
- Fig. 11.—Cross-sections of fibre bundles at the head of ox hide, $\times 330$. The arrows point to the binding reticular tissue. "Neocarmine W."
- Fig. 12.—Rings of Henle in swollen fibre bundles from ox hide $\times 76$.
- Figs. 13-15.—Limed ox hide. Splitting-up decreasing from 13-15, $\times 55$.
- Figs. 16-18.—Limed ox hide. Separation increasing from 16-18, $\times 55$.
- Figs. 19-21.—Limed ox hide. Turgidity decreasing from 19-21, $\times 55$.
- Fig. 22.—Vegetable tanned sole leather from (a) the best part of the bend (the back of the animal); (b) the belly-shoulder corner of the bend; (c) the shoulder, and (d) the belly, $\times 55$.
- Fig. 23.—Belting leather with well split-up fibre bundles, woven at a medium angle of weave, $\times 55$.
- Fig. 24.—Picker band leather showing very fine splitting-up, without separation and a medium angle of weave, $\times 55$.
- Fig. 25.—Gloving leather with good "run," $\times 86$. The fibres are extremely finely split up.
- Fig. 26.—Vegetable tanned sole leather (a) untreated, (b) dried $2\frac{1}{2}$ hours before an electric fire, $\times 15$.
- Fig. 27.—Black semi-chrome shoe upper leather, $\times 55$: (a) Good sample: yellow light; (b) Good sample: near I.R. rays; (c) Damaged sample: yellow light; (d) Damaged sample: near I.R. rays. The fibres can now be seen to be excessively split up and separated.

VII.—MICROSCOPY IN THE INVESTIGATION OF STARCH AND CELLULOSE BREAKDOWN IN THE DIGESTIVE TRACT.

By FRANK BAKER and HAMED NASR.
(Rowett Research Institute, Aberdeen.)

FOUR PLATES AND ONE TEXT-FIGURE.

In a survey of the scope and applications of direct microscopy to the investigation of the breakdown of structural starch and cellulose in the digestive tract five points must be emphasized at the outset.

(1) That micro-organisms play an essential role in the decomposition of cellulosic materials in all, and in many vertebrates an important role in the decomposition of raw starches.

(2) No known artificial culture medium is able to meet equally the growth requirements *in vitro* of all the types of micro-organisms demonstrable *in vivo* in the digestive tract of even a single species of animal ; therefore it is not possible to enumerate the relative numbers of different types of bacteria in a sample by plating-out methods applicable to material containing only a single species whose growth requirements are known. To characterize the microflora of a sample of gut contents by cultural methods it would be necessary either to devise an ideal universal medium capable of supporting equally well the growth requirements of every species of organism in the sample or to have a strictly selective medium for each species of organism capable of supporting full growth of its specific organism and of suppressing that of all the others. To achieve either of these objectives would require a major research offering much tedious labour but little prospect of success in our present state of knowledge. At the outset the best procedure, therefore, is to use microscopy to determine, as far as possible, the types and relative numbers of bacteria present and to isolate for biochemical study such organisms as may be seen to play an important part and for which cultural techniques are available (Baker, 1945, 1946 ; Baker and Harriss, 1948).

(3) Many of the representative microbial species demonstrable *in vivo* have never yet been grown in pure culture outside their place of origin where they normally appear as part of a mixed microbial population (Baker, 1946 ; Baker and Harriss, 1948).

(4) The mixed microbial populations of the digestive tract may show wide and distinctive differences in microbiological composition in diverse animal hosts (Baker and Harriss, 1948).

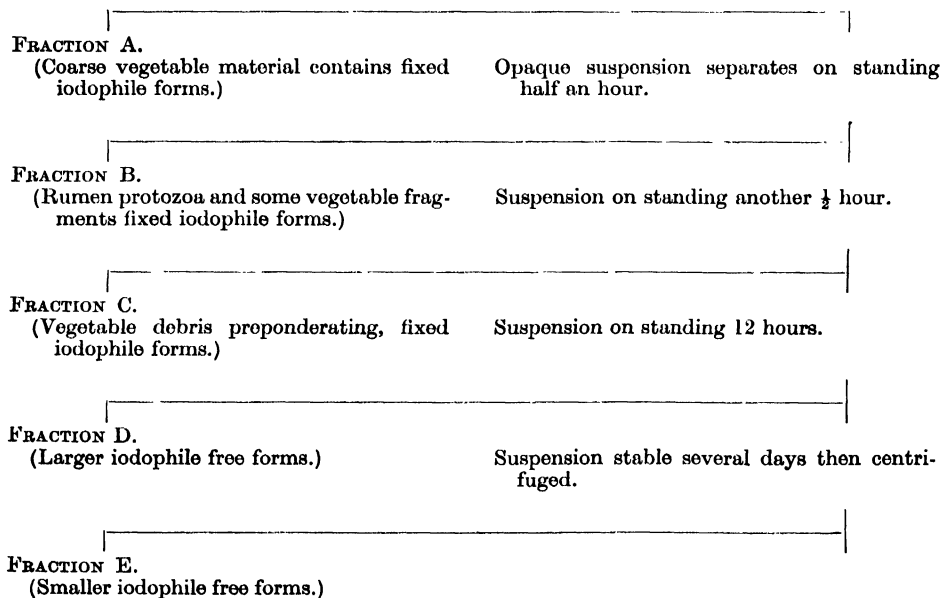
(5) Raw starches and the cellulosic materials normally fed to the animal are neither distinct chemical entities nor mere mixtures but complex systems embracing successive levels and various patterns of gross and fine structural organization.

The role of direct microscopy, therefore, is dual. First, the limitations imposed upon cultural procedures by the divergent growth requirements of a mixed microbial population are so severe that direct microscopic examination of adequately prepared material still offers the most effective means for characterization of the microflora and microfauna of the digestive tract. Secondly, because of the structural integration of the substrate materials, direct microscopy is no less essential for the detailed elucidation of the changes taking place during the progressive dismemberment of the digested vegetable products.

In this account I shall try to show how far these dual requirements have now been met and to indicate how the scope and application of the procedures devised may still be extended. I shall begin, therefore, by dealing with the demonstration of the micro-organisms themselves and of the structural changes attending the breakdown of the cell-wall components of the plant skeleton and raw starch granules.

MATERIALS.

Samples from the rumen, cæcum or other regions of the gut are fixed and preserved immediately after collection in 10 p.c. formalin. Where necessary they are washed and fractionated by straining through muslin followed either by sedimentation or centrifugation at speeds ranging from 500 r.p.m. for coarse up to 10,000 r.p.m. for fine sediments. The following diagram (Barcroft, 1947 ; after Baker, 1943) will illustrate the stratification of fractions occurring in the rumen contents of the ox.



DEMONSTRATION OF MICRO-ORGANISMS.

The protozoa of the digestive tract require no special mention since refined techniques have been elaborated for their demonstration by protozoologists and

are described in all standard texts. Turning, therefore, to the microflora (Schizophycæ, Schizomycetes, Saccharomycetes, Schizosaccharomycetes) it cannot be over-stressed that wet preparations, temporary or permanent, are essential to the demonstration of micro-organisms *in situ* upon cellulosic structures and raw starch granules. Exclusive recourse to dry-film techniques will altogether compromise the success of the investigation. Two simple principles are to be observed in the staining of wet preparations. The first takes account of the fact originally emphasized by W. Henneberg (1922) that, amongst the micro-organisms concerned in the disintegration of starch and cellulose in the alimentary canal, forms giving a blue reaction with iodine are prominent. The reaction is due to the endocellular bacterial synthesis of a polysaccharide resembling starch (Smith and Baker, 1944), and micro-organisms giving this reaction will be termed iodophile, following Heneberg (1922). Iodine solutions, therefore, alone or in combination, are essential routine reagents. Iodophiles, however, are not the only micro-organisms encountered and for the demonstration of aniodophile types recourse must be had to dyestuffs. This brings us to the second point. The majority of aniline dyes employed in the traditional bacteriological techniques stain vegetable residues as strongly as the bacteria themselves, so that it is impossible to obtain clean and contrasted preparations. What is required is a dye with maximal affinity for bacteria and minimal affinity for starch and cellulose. Erythrosin Y. may be employed but is capricious in action and often stains weakly. Aniline Blue is far superior to any of the other dyes so far investigated. Its use was suggested by Professor H. G. Thornton, F.R.S., of Rothamsted Experimental Station, to whom my acknowledgments are due. According to Conn (1946) this dye is a variable mixture of the trisulphonates of triphenyl-p-rosaniline and diphenylrosaniline and is known also as China Blue, Soluble Blue, Cotton Blue, and Marine Blue V. It is important to secure the right brand of dye and the preparation sold by Gurr as Aniline Blue (Water Soluble) is satisfactory.

INVESTIGATION OF CHANGES IN THE STRUCTURAL MATERIALS.

Several types of procedure are available including (a) histochemical, (b) staining, and (c) optical methods. I shall deal successively with these three methods in relation to the direct microscopical investigation of structural cellulose and structural starches respectively.

STRUCTURAL CELLULOSE.

Histochemical Methods.—These are dealt with in a number of standard texts on chemical or botanical microtechnique and require no more than brief mention. It may suffice to recall the detection of cellulose by means of its blue reaction with Schulze's chlorzinciodine, lignin through its red reaction with phloroglucinol and HCl, pectic substances through their strong affinity for ruthenium red, and corky or suberized materials by their action as partition solvents for Sudan III or Scharlach R. Chlorzinciodine is a useful reagent for routine use since it stains the iodophile bacteria themselves as well as the cellulose matrix.

Also since the cellulose reaction is inhibited by the presence of encrusting substances a preliminary histochemical differentiation of the material is effected. Where it is desired to avoid undue swelling, Zimmerman's may be substituted for Schulze's reagent. It is prepared by adding crystalline iodine to a saturated solution of calcium chloride. The intensity of the reaction depends on the concentration of the iodine and may be adjusted to suit requirements.

Staining Methods.—Tetrazonium dyes are employed and amongst them Congo-Red gives a suitable contrast with iodophiles when used in combination with iodine. In combination with Cotton Blue, Bismarck Brown is the most satisfactory stain.

Optical Methods.—Changes in optical properties accompany cellulose breakdown and may be employed to characterize the phases of disintegration of cell-wall structures. In this and other connections I shall deal with the use of the polarizing and the fluorescence microscope.

THE POLARIZING MICROSCOPE.

Since cellulose is birefringent, cellulose structures appear luminous against a dark field under crossed nicols. The maxima and minima of birefringence, moreover, are a function of the internal anisotropy of the microcrystalline matrix. Thus the polarizing microscope has two distinct uses: (i) it gives direct visual information regarding the presence and distribution of cellulose; (ii) indirectly, by observation of the positions of maximum and minimum birefringence, it allows inferences to be drawn concerning the presence and orientation of the micellar chains. The phenomenon of dichroism also deserves attention. When natural cellulosic structures are stained with Congo-red, impregnated with metallic gold by reduction, or treated with chlorzinciodine, microcrystals of dye, gold, or iodine are deposited in the intermicellar spaces (Frey Wyssling, 1935.) Such preparations show colour changes on rotation under a single nicol. With Congo-red, fibres appear colourless when the long axes of the micellar chains are parallel and deeply coloured when they lie at right-angles to the plane of polarization.

FLUORESCENCE MICROSCOPY.

The slight intrinsic fluorescence of cellulose in ultra-violet light is intensified by the presence of certain encrusting substances such as lignin or cutin. The fluorescence microscope may therefore be employed for the detection and histo-botanical characterization of the lignified and cutinized structures which form a large part of the undigested and indigestible residues of natural fodder plant materials. Detection and differentiation may be further facilitated by the observation of the derived fluorescence after pre-treatment with fluorochromes (Haitinger, 1938).

STRUCTURAL STARCH.

It is only very recently that direct microscopy has been systematically applied to the investigation of the microbial digestion of starch so that my account is necessarily tentative and incomplete.

The iodine and cotton-blue methods already described are also available here though the value of iodine is diminished by the intense staining of the granules themselves.

Histochemical Methods.—Iodine is a traditional reagent for the detection of starch itself and of the coloured dextrans produced during enzymic hydrolysis or microbial decomposition. Two further points also deserve attention. Pullki (1938) and others (Jones, 1940; Dadeswell and Wragge, 1940) have shown that the minimum concentration of iodine required to give a colour reaction is not the same for all the granules present in the field or succession of fields examined. Thus, whereas all granules give a reaction at 0.3 p.c. I_2 only a variable proportion react when the concentration is reduced to 0.1 p.c. The actual ratio of reacting to non-reacting granules is affected by mechanical damage. H. Nasr, working with me at the Rowett Institute, has found that the ratio is also changed during the earlier phases of microbial breakdown. Iodine in very low concentrations, therefore, can be usefully employed both for the characterization of the raw starch samples fed to the animal and to determine the onset of breakdown *in vivo* or *in vitro*. Recent investigations (Hopkins, 1946; Geddes, 1946; Schoch, 1947) have confirmed the view originally advanced by Maquenne (1905) that raw starches contain two carbohydrate fractions termed amylose and amylopectin respectively. Also it is now known that the amylose fraction is responsible for the blue reaction with iodine (Hopkins, 1946). Since this reaction is obtained in the presence of so little as 0.1 p.c. of amylose (Hopkins, 1946) it can be employed to demonstrate the selective action of microbial and other agents in the breakdown of structural starches.

Staining Methods.—In comparison with cellulose, the staining affinities of starch for aniline dyestuffs have been little investigated and further research is essential to the design of effective staining procedures. Pullki (1938), Jones (1940), and Dadeswell and Wragge (1940) have shown that intact starch granules do not stain but acquire the capacity to do so as the result of mechanical damage.

Optical Methods.—It is a commonplace that the form and dimensions of starch grains vary with their plant origin. An underlying uniformity of internal structure is disclosed, however, through the presence of the confocal lamellations which surround the hilum. Microscopic examination of the unstained granules affords valuable information concerning the onset and cause of breakdown. Particular attention is given to deformation or indentation of the margins of the granule; swelling; pitting of the surface; and the presence, absence, or deformation of the lamellations.

The Polarizing Microscope.—The intact starch granule is strongly birefringent and examination in polarized light may be employed to detect the occurrence and elucidate the structural features of breakdown. Particular attention is given to uniform or regional changes in birefringence and to the degree of deformation in the extinction quadrants forming the so-called "Cross." It is important to note that birefringence depends on fine-structural integrity of the granule, so that its disappearance does not necessarily indicate chemical change. Thus boiling, which dissociates an easily diffusible amylose fraction from a structural amylopectin residue, destroys double refraction. Recent

researches indicate that birefringence is due to the micro-crystalline orientation within an amylopectin framework of unbranched amylose chains.

ROUTINE TECHNIQUES.

I shall now describe the routine techniques I have used in my investigations on the breakdown in the intestinal tract of structural cellulose and structural starch.

CELLULOSE.

Histochemical.—The customary procedures are adequate and require no further consideration.

Staining.—(1) *Congo-red Iodine Method.*

Stains and Reagents.—(a) Lugol's Iodine ; (b) Congo-red 2 p.c. in 10 p.c. alcohol, boiled to expel CO₂ before using.

Mountant.—Saturated aqueous solution of calcium chloride.

Procedure.—Whole grass blades or other materials taken from silk sacs (Phillipson and Innes, 1939) or metal containers (Asher, 1942) inserted in a fistula animal may be cut into suitable lengths and dealt with in watch glasses. Sediments may be treated by irrigation on the slide.

Congo-red, 15 mins. ;→Water, 2 changes ;→Lugol's Iodine, 1 min. ;→Water, 1 min. ;→Calcium Chloride.

Result.—Iodophiles blue, cellulosic structures pink to red. The structures show dichroism under a single nicol. Preparations last 12 hours.

(2) *Cotton-Blue-Bismarck Brown for Aniodophiles.*

Stains.—Aniline Blue 1 p.c., 2 ml. ; Phenol 5 p.c., 30 ml. ; Glacial Acetic Acid, 8 ml. Allow to stand 1 hour, filter.

Bismarck Brown, saturated solution in 95 p.c. alcohol.

Mountant.—Euparal (jaune).

Procedure.—Aniline Blue, 2 hours at 37° C. ;→95 p.c. alcohol, 2 changes 15–30 secs. ;→Bismarck Brown, 15–30 secs. ;→95 p.c. alcohol, 15–30 secs. ;→absolute alcohol, 5 secs. ;→Euparal.

Result.—Bacteria blue. Cellulosic structures yellow-brown. Permanent.

(3) *Optical Techniques.—Polarized Light.*

Optical.—Microscope with nicol or "Polaroid" polarizer and analyser and rotating stage. Intense filament lamp. A light-green screen may be employed with polaroid to eliminate residual red in the extinction position.

Stain and Mountant.—Stephenson's medium (Lee-Gatenby, 1946). A saturated aqueous solution of potassium iodide is saturated with mercuric iodide and filtered. Two solutions are employed: (i) without, and (ii) with addition of crystalline iodine. With this medium (max. R.I. 1.67) glare is eliminated and definition greatly improved.

Investigation of Starch and Cellulose Breakdown in Digestive Tract. 33

Procedure.—Solution (i), 15 mins. ;→mount in solution (ii). Glass instruments must be employed. The preparation is examined in polarized light under crossed nicols.

Result.—Intact cellulose and cellulosic structures become luminous. Through loss of birefringence accompanying breakdown, the enzymic cavities produced are seen as clear-cut black spaces. On uncrossing the nicols the iodophile bacteria are disclosed *in situ*.

Fluorescence Microscopy.

Optical.—Despite frequent statements to the contrary, quartz condensers are not essential (Haitinger, 1938). Optical glasses are relatively transparent to the low-frequency ultra-violet rays employed in fluorescence microscopy. A convenient light source is an Escura-Ediswan lamp enclosed in a pointolite lamp house. Visible light is filtered off by passage through (i) a Wood's glass screen, and (ii) 10 p.c. copper sulphate solution acidified with sulphuric acid. This solution is placed in a round flask which serves as an auxiliary condenser. The eyes are protected from residual ultra-violet light by a cell containing 10 p.c. aqueous sodium nitrite placed above the eyepiece.

Fluorochromes and Mountants.

Fluorochromes.—Saturated aqueous solution of Thioflavin S. (Gurr) and saturated aqueous Eucrysin R. (Gurr). 10 p.c. of 0.880 ammonia.

Mountant.—Saturated solution of calcium chloride.

Calcium Chloride
/→(for Eucrysin or Thioflavin).

Procedure.—Fluorochrome, 1 min. ;→Water, 2 changes ;

Water (for
→ammonium hydroxide).

Result.—(i) Ammonium hydroxide gives intensification of intrinsic fluorescence for lignified and cutinized structures. Usually : cutin, yellow-white ; lignin, bluish-white.

(ii) Thioflavin S. Intense yellow fluorescence of lignified structures. Cuticle bright blue. A particularly useful combination for routine detection of cuticle in undigested residues of fodder grasses.

Eucrysin R.—Cuticle weakly or not fluorescent. Intact epidermal cells greenish-white. Regions in process of decomposition fiery-orange. Supporting sclerenchyma, bundles and hairs yellow.

STARCH.

The elaboration of specific staining and other techniques for the study of the microbial or enzymic decomposition of structural starch is much to be desired. In the meantime the following notes upon the application of the methods already described must suffice.

Histochemical Techniques.—Iodine and Congo-red solutions are applied by irrigation. Iodide is employed as : (i) Lugol's solution=Iodine, 1.0 g. ; Potassium Iodide, 2.0 g. ; Water, 100 ml. ; (ii) Jones's solutions=(a) Iodine, 0.3 g. ; Potassium Iodide, 0.7 g. ; Water, 100 ml. ; (b) Iodine, 0.03 g. ; Potassium Iodide, 0.07 g. ; Water, 100 ml. Congo-red is employed in 0.1 p.c. aqueous solution.

In Pullki's technique, Iodine and Congo-red are combined as follows : (a) Iodine solution, 10 p.c. Iodine in absolute alcohol ; (b) Congo-red, 1 p.c. in water. 10 g. of gut contents are diluted with water to make 200 ml. of suspension. 0.2 ml. of Iodine is added, followed after 15–30 minutes by 3–5 ml. of Congo-red. Staining is allowed to proceed for 20 minutes and small amounts of sediment are then removed with a pipette and examined under the microscope.

Staining Technique for Micro-Organisms.—When aniline blue is used as a bacterial stain, gut contents are stained in bulk, washed by shaking with water, and then centrifuged down. Such preparations are best examined in water.

OPTICAL TECHNIQUES.

Polarized Light.—Mercuric iodide is unsuitable as a medium for the examination of starch granules since it causes them to swell. Satisfactory results are obtained by dehydrating in bulk with several changes of absolute alcohol centrifuging down, pipetting a small amount of sediment on to a slide, and mounting in Gurr's neutral medium before the alcohol has time to evaporate. When dry the preparation is examined under the polarizing microscope.

APPLICATIONS AND RESULTS.

Cellulosic Structures.—Direct microscopy has been systematically applied to the investigation of cellulosic breakdown in the digestive tract of herbivora and omnivora by Henneberg (1922), Baker (1931–1948 full refs. in Baker and Harriss, 1948), Asher (1942), and Drapala *et al.* (1947).

The accumulated data and permissible inferences have been discussed in detail in a recent review (Baker and Harriss, 1948). Here a summary of the broad conclusions must suffice.

(a) *Features and Detection of Cellulose Breakdown.*

(1) Breakdown is accompanied by simultaneous loss of histochemical reactions and optical birefringence in the affected structures.

(2) The microbial origin of the process of breakdown is demonstrated by the formation of clear-cut enzymic cavities around the responsible micro-organisms.

(3) Actual loss of birefringence and destruction of the micellar framework is preceded by increase of the staining reactions at the periphery of the enzymic cavity.

(4) The rate of breakdown is influenced by the form, surface-volume ratio, and internal surface area of the affected structure (Asher, 1942). The possible influence of the character, dimensions, and mode of organization of the intermicellar spaces on digestibility has recently been emphasized (Baker and Harriss, 1948).

(5) The histological components of fodder-plant materials, such as grasses, are digested in a certain order and at widely different rates.

(6) Lignified and cutinized structures are especially prominent in the undigested residues of fodder plant materials. The presence and distribution of encrusting substances exert a major influence upon digestibility.

(b) *The Microbial Facies of Cellulose Breakdown.*

(1) The decomposition of structural cellulose in the digestive tract is associated with a characteristic microbial facies. By "microbial facies" I mean a constant association in the same natural habitat of several morphologically distinguishable types of micro-organisms.

(2) The facies is influenced by the character of the cellulosic material. In natural fodder-plant materials it includes curved rods and a graded series of coccoids ranging in dimensions from minimum diameters of $0.1-0.5\mu$ to maxima of $2.5-3.0\mu$. The larger coccoid forms give an intense blue-black reaction with iodine. In isolated cellulosic fibres such as cotton wool, filter paper, and delignified pulps, and in regenerated products such as cellophane, the larger iodophile types are absent. The proximate factors controlling the facies are not known; nor is it known whether the forms observed represent pleomorphic variants or an association of symbionts.

(c) *Interaction of Mechanical and Microbial Factors in the Process of Breakdown.*

In herbivora the ingested structural material is subjected to sustained trituration by the molars especially in ruminants. Microscopic examination of appropriate materials enable us to distinguish:—

(1) *An Indirect Effect Consequent upon Gross-Structural Changes.*—In structurally heterogeneous fodder-plant materials trituration is conspicuously associated with fracture and detachment of the cuticle; this, by facilitating permeation of the underlying tissues with rumen liquid, allows free access of bacteria to the exposed cell-wall surfaces. Examination of fistula material demonstrates that crushing greatly hastens the microbial breakdown of structural cellulose in fresh grass blades.

(2) *A Direct Effect Consequent upon Changes Occurring at Finer Structural Levels.*—Such effects had already been observed in chewed ingesta but can be most readily demonstrated by the use of scored cellophane strip (Baker and Harriss, 1948). Microscopic examination of the strip after its insertion into a rumen fistula shows preferential growth of bacteria and increased breakdown along the line of abrasion.

These results afford a secure experimental basis for a radically new approach to the problem of the digestibility of fodder-plant materials in ruminants and non-ruminant herbivora and omnivora, including man. The aim of such an approach must be to restate the problem of digestibility in structural terms and to correlate the gross- and fine-structural organization of the cellulosic materials fed to the animal with the degree of maturity, regional characteristics and manurial status, of different fodder-plant species. Investigations specifically directed to this end are now in progress at the Rowett Institute.

STRUCTURAL STARCH.

The lower animals commonly ingest dietary starch as an uncooked structural product. Direct microscopy, therefore, besides being essential to the elucidation of the structural and histochemical features of breakdown, enables the onset of distintegration to be detected, its site located, and its progress followed throughout the length of the digestive tract. The method cannot be applied to cooked food since boiling destroys the structure of the granules. The work of Kon, and Porter (1947-1948) on refectation and recent researches on ruminant digestion have established that under certain circumstances microbial action can play a role in the breakdown of starch not less important than that of the digestive secretions of the animal. Direct microscopy, therefore, can be usefully employed in characterizing the micro-organisms responsible for this process. It is worth noting that the extensive literature dealing with the bacterial decomposition of starch (summarized by Thaysen and Galloway, 1930, and Porter, 1946) is largely based upon cultural investigations in which soluble starch was employed as a substrate. Raw-starch granules are far less readily broken down, so that results with soluble starch afford an inadequate or misleading picture of the changes in uncooked diets. Direct microscopy is essential because many of the bacteria responsible for starch decomposition have not yet been isolated in pure culture; even with bacteria that can be grown, the uncertainty of success and time-consuming character of the requisite techniques prohibit their use in the routine examination of large numbers of animals; although cultural investigations combined with direct microscopy are essential to full understanding of bacterial decomposition of starch and its nutritional consequences. Direct microscopy was first applied to the study of starch breakdown in ruminant digestion by the Ondesterpoort school, notably Van der Wath and Myburgh (1941). Subsequently I was able to apply similar methods to several non-ruminant species (Baker and Harriss, 1948). The results of these earlier investigations form the point of departure for a comprehensive programme of work upon the breakdown of structural starches now under way at the Rowett Institute. The aims of the investigation are to ascertain: (1) the histochemical and histophysical changes in structural starches during passage through the digestive tract; (2) the region of the gut in which these changes occur for a single type of starch in different animals and for different types of starch in the same animal species; and (3) the relative contributions of the digestive secretions of the animal and the microbial population of the gut in the breakdown of structural starches.

In summarizing the results so far available from these investigations I shall deal with the uniform structural and histochemical features of breakdown and then in general terms with the circumstances governing their appearance.

Structural and Histochemical Features of Breakdown.

Breakdown, whether due to microbial agencies or digestive secretions, is accompanied by progressive changes in the morphology staining and histochemical reactions and optical behaviour of the granule. In the initial stage the reaction to strong (Lugol's) iodine is unaltered but with dilute (Jones b) iodine

there is a loss of intensity and a reddish purple replaces the blue colour observed with intact starch. At a later stage, the reaction to strong iodine is also diminished and changes from black, through purple-brown and brown-purple intermediates, to red-yellow or yellow-red shades. The reaction contours are irregular and the changes may commence either at the hilum or the periphery. This phase is associated with decrease in birefringence, which, like change in iodine reaction, is irregular and gives the granule a fragmented appearance in polarized light. At this stage swelling is comparatively inconspicuous but, where disintegration is due to bacterial action, the margins of the granule may be markedly indented or the whole granule fractured. The micro-organisms may form a dense layer several cells thick entirely surrounding the granule. Those immediately attached to the surface are situated within defined enzymic cavities whose formation causes extensive pitting. If the iodine reaction is exclusively (Hopkins, 1946 ; Geddes, 1946 ; Schoch, 1947) and the birefringence chiefly (Schoch, 1947) attributed to the presence of well orientated unbranched amylose chains, these observations show that decomposition of the amylose fraction precedes destruction of an amylopectin residue. The persistence of lamellations and faint extinction quadrants in these non-reacting residues is in agreement with the view of Meyer (1940) that a branched amylopectin framework sustains the structural integrity of the granule. The final phase of disintegration is accompanied by disappearance of the lamellations, complete loss of birefringence, and marked swelling which culminates in the dissolution of the residue.

Dietary and Physiological Circumstances Affecting Breakdown of Starch.

Using these features as indicators of microbial or enzymic breakdown it has been found :—

(1) That the region of the digestive tract in which breakdown occurs depends both on the gut architecture of the animal under investigation and the type of starch present in the diet.

(2) Thus in the same species there may be different sites of breakdown for different types of starch, and for the same type of starch there may be different sites of breakdown for various animal species.

(3) The relative roles of microbial agents and digestive secretions in effecting breakdown depends upon the site of breakdown of the type of starch fed to the animal.

(4) In each of the animal species so far investigated breakdown is associated with the development of one of a limited number of distinctive types of microbial facies. The type of facies tends to remain constant for a given species.

(5) Iodophile bacteria predominate in all the types of facies so far encountered. Thus, in general, the microbial breakdown of dietary starch is accompanied by synthesis of bacterial polysaccharide.

More detailed discussion of these results would be out of place on this occasion. They will be dealt with in detail in a paper to be prepared in collaboration with H. Nasr and F. Morrice.

Future Scope and Applications.

The future applications of direct microscopy in this field may best be considered in regard to its correlations with other methods of investigation. Since the potential correlations are numerous I shall confine myself to certain aspects of a single topic—namely, the technical prospects of a structural approach to the problems of digestibility.

As I have stressed throughout, in considering the digestion of the cell-wall components of the plant skeleton, we are dealing with progressive phases in the dismemberment of a structural edifice. The spatial levels of this edifice constitute what Woodger (1931) and Needham (1936) have described as a "hierarchical organization" and may be indicated schematically as follows:—

Gross Anatomical Level (Molar-macroscopic), e.g. the entire grass blade.

Histological Level (Molar-microscopic):—

- (a) Tissues, e.g. the epidermis;
- (b) Tissue components, e.g. a bast fibre.

Micellar Level (Submicroscopic), e.g. individual cellulose chains.

Molecular Level, e.g. the β glucosidic components of the cellulose micelle.

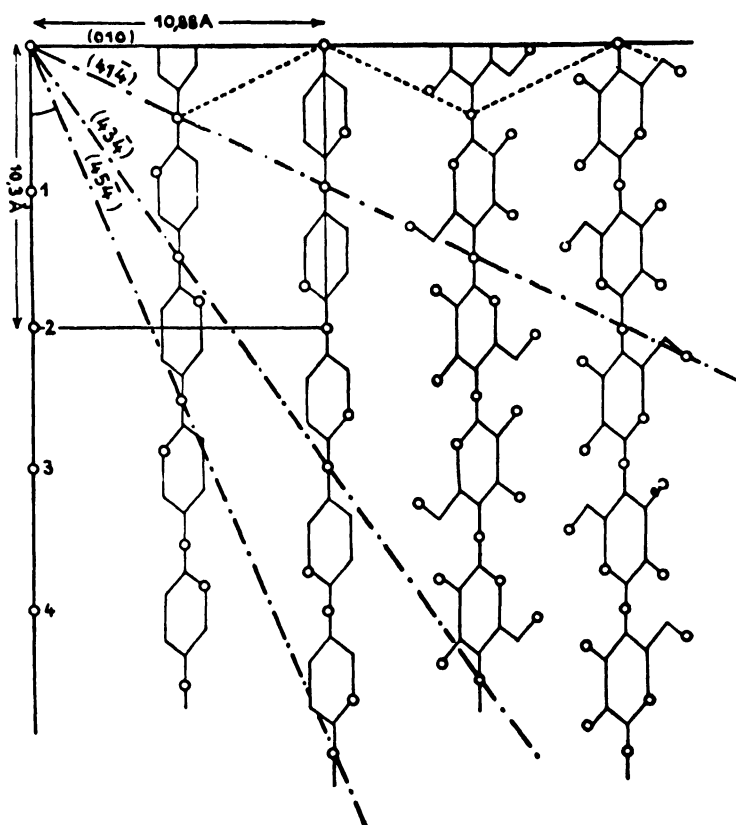
Sub-Molecular Level, e.g. the oxygen linkages of the main valence chains.

The investigation of these levels may proceed in two directions. Thus attention may be focused upon the indefinite extension of technical procedures requisite to the exploration of a single level. Alternatively, we may deal with each level in relation to the levels above and beneath it. Here the domains of specialized techniques overlap and it is at these frontiers, I should like to suggest, that the potentialities of the future reside. A case in point is the use of the polarizing microscope in the investigation of structural breakdown. Under normal illumination the compound microscope affords a direct visual image of structure within the limits imposed by the magnification and effective resolving power of the optical system. By the introduction of polarized light, its range is indirectly extended to include, through inferences drawn from extinction maxima, for example, the underlying submicroscopic organization of the micellar-molecular levels. These inferences overlap with and can be controlled by reference to others drawn from the observed features of X-ray diffraction spectra. Also, both types of inference may be checked and still further elucidated by reference to the structural image directly presented upon the screen of the electron microscope. In this way direct microscopy is brought into working reciprocity with the newer techniques employed in fine structural research. The conclusion appears inevitable, therefore, that further progress in determining the role of the structural factor in digestion will in large measure depend upon the systematic application of polarization-optical techniques and equipment to the routine microscopic examination of gut contents and dietary products.

This view carries a number of corollaries. The intrinsic birefringence of structural starch and cellulose is a consequence of microcrystalline organization at the micellar-molecular levels. But it is known that the optical anisotropy of a crystal is associated with other directional features including anisotropies of

form, cohesion, thermal conductivity, growth, solution, and chemical reaction (Rinne, 1922). Such correlations, first established for mineral crystals, are shown by micellar-molecular as well as atomic and ionic lattices. Some actual examples will point the issue. By the use of the polarizing microscope Maby (1936) established "a precise correlation between percentage longitudinal shrinkage, the mean slope of spiral slits, and microscopic fibrillæ in sixteen species of coniferous woods." He referred this correlation to the orientation of the micellar chains, whose actual inclination to the axis of the conifer tracheide had in the meantime been independently determined by Preston (1934) through a combined application of polariscopic and X-ray diffraction analysis. Through Preston's work, moreover, the dependence of the micellar inclination itself upon the growth and elongation of the cambial initials was established. Nearly at the same time Bailey (1937) reached analogous conclusions in regard to the influence of fine structure in determining the form and orientation of the enzymic cavities produced during the fungal decomposition of coniferous wood. Finally, Frey-Wyssling showed that the directional hydrolyses observed by Bailey proceeded along predetermined planes of the cellulose chain lattice as computed from X-ray diffraction analysis. The accompanying diagram taken from Frey-Wyssling's paper (1938) illustrates these features.

Such results by establishing a sequence of correlations between gross- and fine-structural organization, physical anisotropy, and the directional features of



growth and breakdown, foreshadow the formal outlines of a scheme of relationships which embraces the influence of genetic and non-genetic factors upon the digestibility of fodder-plant materials as a special case. The theoretical consequences which follow from this premise are manifold and need not be elaborated here. The practical conclusions which can be drawn are more obvious and I shall confine attention to them. For the postulated correlation implies that the methods used and the results obtained in several adjacent fields of investigation are in fact interchangeable. For example, when for economic or other reasons wood pulps are fed to domestic animals the information originally assembled in the interests of the timber and paper industries can often afford direct insight upon the factors determining the relative digestibility of different products. In these industries, moreover, as also in textile technology, the compound microscope with normal illumination or polarized light has been systematically employed in conjunction with X-ray diffraction analysis to determine the conjoint influence of all levels of structure upon the strength and physical properties of materials. It is certain, therefore, that the structural approach to digestibility may profit from the example and derive the greatest advantage through a wholesale appropriation of techniques and data from these more advanced branches of investigation. This exchange may in the interval be facilitated by the inclusion of available data and affiliated techniques from the fields of cereal chemistry and the microscopical study of drugs and food products. The foregoing account of the present applications of direct microscopy to the study of starch and cellulose breakdown in the digestive tract is intended as a preliminary effect toward the ultimate achievement of these ends.

SUMMARY.

This paper describes in detail a range of microscopic techniques used in the study of starch and cellulose breakdown in the digestive tract.

Reasons are given for undertaking work aimed at expressing digestibility of fodder plants and other nutrients in terms of their accessibility to bacterial disintegration and an outline is given of some results already observed.

ACKNOWLEDGMENTS.

My thanks are due to my assistant Mr. F. Morrice and also to Mr. W. G. McLeod for expert assistance in the preparation of photomicrographs and lantern slides. My attention was first called to the use of direct microscopy in the study of cellulose breakdown by Dr. A. C. Thaysen and it is a pleasant duty to acknowledge the help and encouragement I have received from him for many years.

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DESCRIPTION OF PLATES I TO IV.

- Fig. 1 (*a, b*).—Epidermal cells of grass blade taken from rumen of sheep. Polarized light technique (*see* p. 34) for demonstration of breakdown of natural cellulosic materials through loss of birefringence. Preparation mounted in mercuric-iodide solution with addition of iodine. (*a*) Nicols crossed showing loss of birefringence (black) in enzymatic cavity; (*b*) Nicols uncrossed showing large iodine reacting bacteria (iodophiles) *in situ*. ($\times 800$ app.)
- Fig. 2 (*a, b*).—Scored cellophane after 24 hours in rumen of a fistulated sheep. To show use of cotton-blue staining technique (p. 32) in demonstration of micro-organisms (iodophile or aniodophile) concerned in breakdown of natural or artificial cellulosic materials. The preparation shows preferential growth of micro-organisms along the scratched line. (*a*) General effect low power ($\times 100$); (*b*) High power view ($\times 500$) showing individual groups of micro-organisms. Mounted in Euparal.
- Fig. 3.—Grass-blade (Yorkshire fog) after 48 hours in rumen of fistulated sheep. To show use of fluorescence microscope in the rapid histobotanical characterization of undigested (usually cutinized or lignified residues). The plant hairs and vascular tissues (xylum and associated sclerichyma) resist digestion and fluoresce strongly. Preparation mounted in dilute ammonium hydroxide. ($\times 100$; *see* p. 33.)
- Fig. 4.—(Same as fig. 3.) Advanced stage of breakdown. Complete microbial faecies of iodophiles (*see* p. 37) and extensive formation of enzymatic cavities. ($\times 800$.)
- Fig. 5.—(Same as fig. 3.) Higher magnification ($\times 1500$). Showing three giant iodophiles and edges of enzymatic cavities.
- Fig. 6.—Grass blade taken from rumen of sheep. Epidermal cells. To show use of Congo-Red-Iodine technique (*see* p. 32) for contrast staining of iodophiles and cellulosic structures. Large iodophiles are seen at the tips of finger-like enzymatic cavities formed on the cell-surface. Mounted in sat. sol. of calcium chloride. ($\times 800$.)
- Figs. 7–9.—Potato-starch granules to show use of polarized light in the detection through loss of birefringence of breakdown of starch in the digestive tract. (1) Intact potato starch. (2) Regional loss of birefringence in starch granule in caecal contents of pig fed potato-starch diet. (3) Later stage of breakdown (H. Nasr). Mounted in Gurr's medium. ($\times 500$.)
- Fig. 10.—Potato-starch granule from caecal contents of pig to show demonstration of adherent iodophile micro-organisms (*Clostridia*) by use of iodine solution (H. Nasr). ($\times 500$.)
- Fig. 11.—Potato-starch granule from caecal contents of pig to show use of iodine for demonstration of breakdown of granule (advanced). Numerous iodophile bacteria (*clostridia*) in interior. ($\times 800$.)
- Fig. 12.—Potato-starch granule from pure culture of *Clostridia* from caecum of pig to show use of iodine solutions in demonstration of histochemical features of breakdown. Breakdown is accompanied by progressive regional disappearance of the blue-reacting (amylose) component of the granule leaving a non-reacting structural residue (presumed to be partially dextrinized amylopectin). ($\times 500$.)
- Fig. 13.—Plant hair from epicarp of barley undergoing fungal decomposition in the voided faeces of a horse. Polarized light. To show the formation of enzymatic cavities with well-marked angular apices as described by Bailey and Kerr (1937) and interpreted in terms of the fine structural organization of the cellulose micelle by Frey-Wyssling (1938). ($\times 500$.)
- Fig. 14.—Potato-starch granules from old culture of *Clostridium* isolated from caecum of pig showing various phases in removal of blue-reacting (amylose) component accompanied by production and extensive swelling of the non-reacting residue (presumed to be partially dextrinized amylopectin). A few intact granules are introduced for purposes of comparison. ($\times 200$.)



Fig. 1a.



Fig. 1b.



Fig. 2a.



Fig. 2b.



Fig. 3.



Fig. 4.



Fig. 5.



Fig. 6.

[To face Plate III.]



Fig. 7.



Fig. 8.

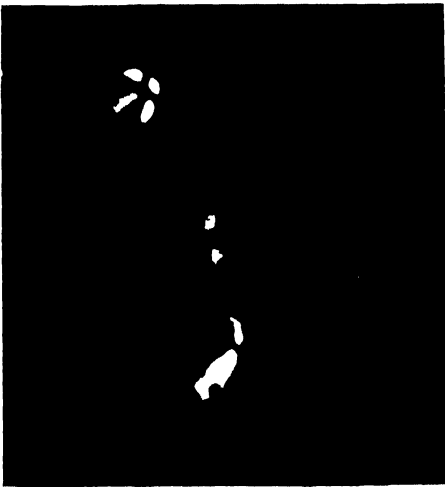


Fig. 9.



Fig. 10.

[To face Plate II.]



Fig. 11.



Fig. 12.



Fig. 13.



Fig. 14.

VIII.—HABITUATION.

By EUGÈNE PENARD.

THREE TEXT-FIGURES.

IN the Proceedings of the Linnæan Society 1943-4, under the title "Some Problems of Animal Learning," W. H. Thorpe has given results of his researches into what he calls Habituation ; a term which might be explained as follows :

An animal, when some danger threatens, very quickly puts itself into a state of defence—by flight, by the sudden closure of its shell, or in some other way. Then one may observe that after a certain number of knocks on the table upon which the microscope is standing for example, the animal, frightened at first, is satisfied that the blows are after all not in the least dangerous. It is so far reassured that it no longer remains in hiding, but expands yet again and remains so without further response to the stimulus.

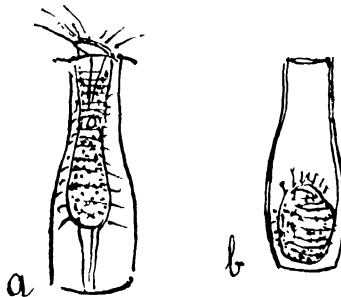


FIG. 1.

Metacystis lagenula. (a) Extended. (b) Retracted.

Thorpe has chosen examples from a wide field, from Mammals to Coelenterates ; but he has not referred to the Protozoa at all. They are too simple, he may think, having no nervous system. But that would be a mistake on his part. The Protozoa also may show such behaviour. They also may remain extended in spite of a repetition of blows which at first caused them to retreat quickly into their shelters. Long ago I myself described something of the kind in an Infusorian such as *Metacystis lagenula* (see fig. 1), and in a Heliozoon *Heterophrys glabrescens* (see fig. 2). And even in a shelled Rhizopod *Hyalosphenia cuneata* I imagine there would be the same sequence of events (see fig. 3).

Metacystis lagenula.—"One day I came upon a specimen of *Metacystis lagenula*, if I am not mistaken, which was particularly favourable for observation ; my notes are now lost and it may have been another Infusorian. I began to knock the table on which my microscope was standing and the animal

at once shot back into its shell, but almost immediately stretched out again. I knocked again ; in it went and then came out ; and for a long while I continued repeating the experiment. But—and this is the important point—little by little its responses lost their intensity, until finally they ceased altogether, and the animal remained extended quite undisturbed by what I was doing.” *

Heterophrys glabrescens.—“The phenomenon of retreat may be studied in this species with particular ease, the animals responding rapidly and several times in succession, up to six times or even oftener. But at each repetition the

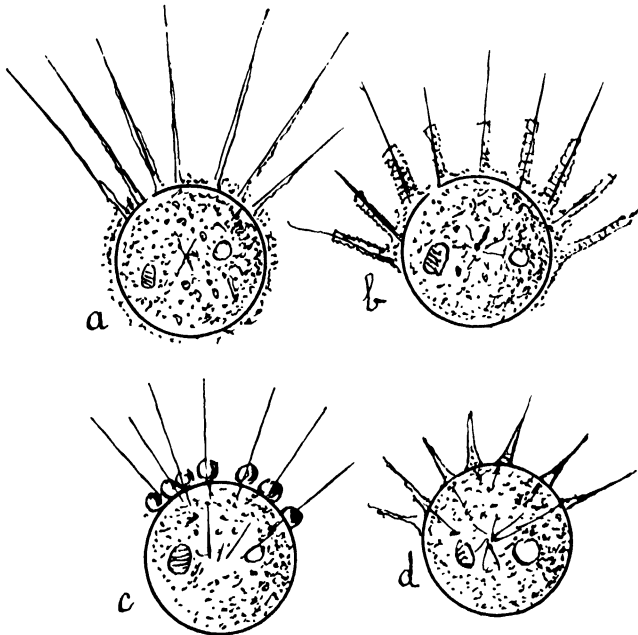


FIG. 2.

Heterophrys glabrescens. (a) Expanded. (b) Sudden contraction. (c) Complete contraction. (d) Re-emergence.

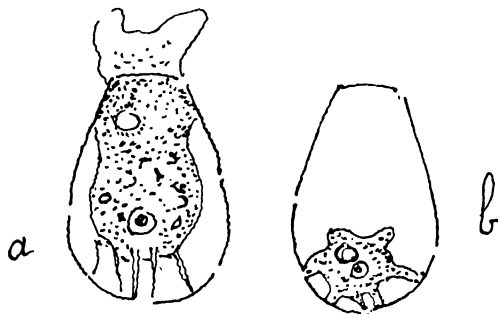


FIG. 3.

Hyalosphenia cuneata. (a) Expanded. (b) Sudden contraction.

response is more difficult to elicit, the blows on the slide must be ever stronger, and even so in the end the reaction fails." *

(In the figures the pseudopodia are shown only over a part of the circumference ; actually of course they arise all over the surface of the spherical body.)

Hyalosphenia cuneata.—Perhaps I have no right to associate this species with the phenomenon of Habituation. Although I have certainly on many occasions witnessed its sudden withdrawal into the fundus of its shell, followed by a speedy extension, its movements are so much slower that I grew tired of watching. Later on I could not find my Rhizopod again, but I certainly imagine it also would exhibit habituation.†

* "Héliozaïres d'eau douce," 1904, p. 164.

† See "Faune rhizopodique du Bassin du Léman." Geneva, 1902, p. 335.

ABSTRACTS AND REVIEWS.

TECHNICAL MICROSCOPY.

Interference Microscopy.—E. J. AMBROSE ("An Interference Microscope for the Study of Inhomogeneous Media," *J. Sci. Instr.*, 1948, **25**, 134–5). A modification of the Fabry-Perot interferometer is described which can be attached to a standard microscope and used to measure small localized changes of refractive index within the specimen. The latter is mounted between two partially reflecting glass plates and is illuminated by light from a small aperture in a plate fixed in the focal plane of a 16 mm. microscope objective, used as a collimator. The method is probably not so sensitive as phase-contrast for objects not showing more than one fringe, but is very suitable for studying microscopic detail in objects covering a large part of the field of view. In the case of irregular objects of uniform refractive index the image is a true two-dimensional representation of a three-dimensional object. The method has been applied to the investigation of the concentration gradients produced by the diffusion of a liquid into a transparent solid. Differences of refractive index of one part in 10,000 can readily be measured.
B. O. P.

Phase-contrast Microscopy.—R. BARER ("Some Applications of Phase-contrast Microscopy," *Quart. J. Microscop. Sci.*, 1947, **88**, 491–500). The simple theory of phase-contrast microscopy can be extended without difficulty to cover such cases as stained objects in which absorption and retardation occur simultaneously. The thickness of the object is not important, it is the phase-change produced and transparency which matter. Objects suitable for study by the method include Chaoborus, Chironomus and young mosquito larvæ in the living state. Examples of stained objects reproduced in the plates include an Epididymis tubule; Liver in pernicious anæmia; Purkinje cells from the cerebellum, and Melanoma of the skin. In all cases details are revealed in the phase-contrast image which are absent when normal illumination is used. The method should be employed with caution but may prove to be a valuable adjunct in the routine examination of normal and pathological stained sections.
B. O. P.

A Leeuwenhoek Microscope.—E. FRISON ("A Leeuwenhoek Microscope. An Account of the Almost-forgotten Instrument in the Henri van Heurck Collection at the Natural History Museum, Antwerp, with a brief Historical Survey," *The Microscope*, 1948, p. 1). The author, who is microscopist to the City of Antwerp, gives a description of the Leeuwenhoek microscope now in the Henri van Heurck Collection in the Natural History Museum at Antwerp. This is one of the six one-lens microscopes still remaining out of the ninety-five which were sold at Delft in 1747 after the death of Maria, Anthony van Leeuwenhoek's only daughter. The Antwerp specimen has a lens 1.09 mm. thick with a focal length of 2.50 mm.: its resolving power stops at the first group of lines of the Nobert Test Plate (443 lines per millimetre). Six excellent photomicrographs taken with the microscope are reproduced. The twenty-six microscopes of the Royal

Society which were bequeathed by van Leeuwenhoek have of course disappeared, after having been borrowed by the surgeon Sir Evered Home. What, however, has been the fate of the two microscopes bequeathed to Queen Mary, the wife of William III? Are these microscopes still to be found buried in the Royal Collections? G. M. F.

Photomicrography.—O. W. RICHARDS ("Phase Photomicrography," *J. Biol. photogr. Assoc.*, 1947, **16**, 29–38). Phase-contrast microscopy is useful for the examination of transparent materials with small optical path differences or low absorption contrast, such as living organisms and stained specimens of low contrast. Various types of diffraction plate are discussed, their applications are given and the Spencer equipment is briefly described. Ortho or process emulsions are suitable for the phase photomicrography of uncoloured specimens whilst, when colour is present, a panchromatic emulsion is to be preferred. Pictures in full colour may be made with Anscoolor or Kodachrome. The exposure necessary depends upon the diffraction plate and when plates of low transmission are employed may have to be increased up to about four times that required for the same objective used normally with the condenser closed enough to give optimum contrast. The use of photo-electric exposure meters is discussed and methods for making stereophotomicrographs are suggested. A fast flash discharge tube source may be used to stop locomotion and Brownian movements of the specimen. A simple, visual, step photometer is described for estimating the required exposure.

B. O. P.

Phase-contrast Microscopy.—O. W. RICHARDS ("Biological Phase Microscopy," *Cold Spring Harbour Symposia on Quantitative Biology*, **9**, 208–14). The history of the development of phase-contrast microscopy is outlined with full references. The mode of operation of the system is described with the aid of an optical diagram and a system of nomenclature useful for the classification of various types of diffraction plate is evolved. Bright contrast is usually preferred for counting and dark contrast, in which the image more nearly resembles that obtained from a stained preparation, for measuring. When the optical path differences in the specimen are large dark field microscopy is indicated and phase-contrast microscopy is preferable when the path differences are small. Suitable sources of illumination and methods of mounting specimens are described. Applications are given and results obtained upon various types of material with many kinds of diffraction plate are listed. In general, the quarter-wave retardations have proved most useful, but other retardations are better for some specimens. Several aspects of photomicrography with the phase-contrast microscope are dealt with and a representative series of photographs is included.

B. O. P.

Oblique Illuminator.—R. F. SHROPSHIRE ("Oblique Photomicrographic Illuminator," *Trans. Amer. Micr. Soc.*, 1947, **66**, 269–71). A device is described which enables a high intensity source, such as is normally used for the photomicrography of transparent objects, to be employed for the oblique illumination of opaque specimens. The light source remains in position on the optic axis and the stage is side-stepped by means of an arrangement of a periscopic pair of prisms in conjunction with a concave mirror. Obliquity of illumination can be controlled and, if known, permits the heights of objects to be determined by measuring the lengths of the shadows cast by them.

B. O. P.

HISTOLOGICAL AND CYTOLOGICAL TECHNIQUE.

The Feulgen Reaction in Block Staining.—J. F. LHOTKA and H. A. DAVENPORT ("Differential Staining of Tissue in the Block with Picric Acid and the Feulgen

Reaction." *Stain Technol.*, 1947, **22**, 139-144, 8 refs.). Pieces of fresh mammalian tissue not thicker than 5 mm. are fixed for approximately 48 hours at 25° C. in a mixture of equal parts of 5 p.c. aqueous sulphosalicylic acid and saturated aqueous picric acid. Tissues are washed for 30 minutes in three 10-minute changes of distilled water and placed in Feulgen's staining solution diluted to one-half strength with distilled water. The staining solution is allowed to act for 24 hours on blocks of 2 to 3 mm., up to 48 hours for blocks of 5 mm. thickness. After staining, the specimens are transferred to a mixture of sodium bisulphite 0.5 gm. and N. hydrochloric acid, 5 ml. in 100 ml. of distilled water. Two changes of 15-30 minutes each in the acid sulphite solution are given and these are followed by dehydration through 50, 70, and 95 p.c. alcohol. One or two hours are allowed for each change except the last 95 p.c. in which the stained tissue remains overnight. To complete the dehydration two changes in absolute alcohol are followed by clearing in xylol and embedding in paraffin. Sections are cut of 10 μ or less, placed on slides, the paraffin removed and mounted. Nuclei are stained reddish violet against a lemon-yellow background when the stain is typical Orange G, 200 mgm. per 100 ml., may be added to the fixing fluid if a more polychromatic effect is required.

G. M. F.

Oil Soluble Dyes for Orange Vesicles.—G. S. KING ("Some Oil-soluble Dyes which Stain Suberized Deposits in Orange Vesicles," *Stain Technol.*, 1947, **22**, 145-7, 6 refs., 1 fig.). Of sixteen commercial oil-soluble dyes, not previously in common use among botanists, eight have proved better than Sudan III and Sudan IV in staining the suberized areas of the fruit vesicles of the orange. Oil blue NA which is already known as a stain for rubber is effective while the other dyes are oil red XO, oil red OB, Calco oil red, alizarine irisol base, spirit blue, Calco orange SS and oil yellow AB. These stains are more brilliant and deeper than the Sudan dyes.

G. M. F.

Squashing and Mounting after any Stain.—J. A. SERRA ("A Simple Method for Squashing and Mounting Preparations after any Stain," *Stain Technol.*, 1947, **22**, 157-9). The smearing or squashing method (Nuklealquetschmethode) after Feulgen staining is used for chromosomes. Small pieces of tissue (0.5-1 mm.) are stained after suitable fixation in hæmatoxylin or coal-tar dyes, washed thoroughly, squashed or smeared in glycerin. The glycerin is slowly removed with water by capillarity and the water is afterwards replaced in the same way by alcohol. The cover-glass is then removed and after a rapid rinsing in alcohol, the materials are permanently mounted in alcohol-soluble resin.

G. M. F.

The Stability of Acid Phosphatase.—A. M. LASSEK ("The Stability of So-called Axonal Acid Phosphatase as Determined by Experiments in its Stainability," *Stain Technol.*, 1947, **22**, 133-8, 2 refs., 6 figs.). Gomori (*Arch. Path.*, 1941, **32**, 189) introduced a method for the detection of acid phosphatase which is thought to depend on the action of the enzyme in splitting the phosphate radical from a suitable substrate (sodium glycerophosphate) contained in a solution having an acid buffer mechanism and lead nitrate. So-called acid phosphatase in spinal cord axons cannot be inactivated by various fixing solutions, changes in pH, and post-mortem autolysis, nor by well-known enzyme inactivators. It thus seems likely that Gomori's staining method depends on factors other than enzyme activity.

G. M. F.

Paraffin Embedding.—A. S. FOSTER and E. M. GIFFORD, Jr. ("Improvements in the Paraffin Method," *Stain Technol.*, 1947, **22**, 129-132, 8 refs.). Certain plant and animal tissues are difficult to section after being embedded in paraffin. Exposure of

woody tissues to hydrofluoric acid produces a partial degradation of cellulose with formation of hydrocellulose.

Three fluids of differing composition were employed.

Schedule.	Composition of fluid.	Exposure at 22° C. .
1	10-15 p.c. hydrofluoric acid.	3.7 days.
2	20 ml. glycerin ; 80 ml. 70 p.c. ethyl alcohol ; followed by 10 p.c. hydrofluoric acid.	1 week. 3.7 days.
3	10 ml. glycerin ; 10 ml. hydrofluoric acid ; 80 ml. 95 p.c. alcohol.	3 days to 2 weeks.

The softening action may be accelerated by conducting the process at 32° C. All schedules are satisfactory but 3 is recommended for general use. The presence of tannins and phlobaphene compounds may interfere with sectioning. The following solution removes these compounds :

Chromic acid	..	..	..	..	..	..	1 g.
Potassium bichromate	..	..	..	..	..	..	1 g.
Glacial acetic acid	..	..	..	..	..	..	10 ml.
Distilled water	..	..	..	..	..	..	90 ml.

Depending on the material, 12 to 48 hours is adequate. Sections must be washed in running water for 1-2 hours before staining. G. M. F.

Staining Stomatal Starch.—O. V. S. HEATH (" Role of Starch in Light-induced Stomatal Movement, and a New Reagent for Staining Stomatal Starch," *Nature*, **159**, 647-8). The reagent is prepared by dissolving 2.5 g. of iodine in 100 ml. of phenol, containing the minimum amount of water to make it liquid at room temperature and an excess of potassium iodide. As the refractive index of this reagent is very high excellent images may be obtained by mounting the material in it. G. M. F.

Chlorazol Paper Brown B.—B. VERDCOURT (" Chlorazol Paper Brown B as a Stain for Plant Tissues," *Stain Technol.*, **22**, 155-6). Chlorazol paper Brown B is used in a saturated aqueous solution, the solubility being about 30 g. per litre at 14° C. Material may be stained overnight but if the delicate cell contents are not required for study boiling in the aqueous stain for one or two minutes will produce an equivalent depth of staining. The material is then differentiated in 1 p.c. nitric acid solution. Dehydration in acetone and mounting in Euparal are best for preserving the colours. By the boiling technique finished slides of *Clematis vitalba* L. were obtained in five minutes. The slides retain their tints for at least a year. The tissues and their respective colours are : Epidermis, yellow ; Cortex, yellow ; Pericycle (lignified tissues), blood-red ; Xylem, salmon to blood-red ; Phloem (primary), orange ; Cambium, plane yellow ; Phloem (secondary), crimson ; Sieve plates, crimson or orange ; Pith, Amber. Monocotyledon tissue, animal sections, and fungi are not stained.

G. M. F.

Staining *Treponema pallidum*.—H. A. COHEN (" A New Quick Method for Staining *Treponema pallidum*," *Acta med. orient.*, 1947, **6**, 99-100). A thin film must be fixed immediately in 36-40 p.c. formalin, which is then heated till evaporation occurs. The film is then washed with distilled water. Staining is carried out by flooding the film with four drops of a solution of equal parts of N/1 NaOH and N/10 NaOH followed by

two drops of a 5 p.c. ferric chloride solution, four drops of N/1 hydroxylamine hydrochloride and three drops of a 10 p.c. solution of pyrogallol acid. The film is then warmed till bubbles appear when the solution is stirred at the same time. The slide is rinsed with distilled water after which a little ammoniacal solution of silver nitrate is added (0.5-5 p.c. solution). The film is washed and dried when *T. Pallidum* is a reddish-brown colour.

G. M. F.

Phloxine as a Histological Stain.—R. ROSENBAUM ("Phloxine as an Histologic Stain, especially in Combination with Hæmatoxylin," *Stain Technol.*, 1947, **22**, 149-53, 17 refs.). Phloxine can be used as a counterstain to Ehrlich's acid hæmatoxylin. Tissues are best fixed in Zenker's fluid, although formalin can be used. After embedding in paraffin and cutting sections at 8-10 μ the sections are mounted on albuminized slides. Sections are placed in xylol for 1 minute and then in two changes of 95 p.c. alcohol for 1 minute each. Sections are then washed in tap-water and placed in iodine-alcohol for 3 minutes to remove mercuric chloride crystals. The iodine colour is bleached by a weak solution of sodium hypochlorite. Sections are placed in Ehrlich's acid hæmatoxylin for 7 minutes and well washed in tap-water. The solutions stain better if a good washing is given after each of the above steps. The sections are then placed in Scott's bluer (Potassium bicarbonate, 2 g.; magnesium sulphate, 20 g.; distilled water, 1 litre) for 30 seconds and washed well. The sections are dipped in a 5 p.c. solution of acid alcohol and washed quickly in tap water. The slides are then placed in a 5 p.c. (aqueous) solution of phloxine for 10 seconds. The dye gives a pink colour to the cytoplasm. Sections are then washed thoroughly in tap-water and carried through 95 p.c. and absolute alcohol. The slides are then placed in equal parts of xylol and creosote and this intensifies the stain. Clarite is the best mounting medium. Nuclei are medium purple against a light pink medium.

G. M. F.

Staining Distemper Inclusions.—J. R. GORHAM ("Pollak's Trichrome Stain for Demonstrating Distemper Inclusion Bodies in Tissue Sections," *Science*, 1947, **107**, 175, 2 refs.). Any standard fixative can be used. After sectioning and bringing down to water the sections are stained in Weigert's iron-hæmatoxylin for 4-8 minutes, after which they are washed in running water for the same length of time. They are then stained in Pollak's trichrome stain (Pollak, O. J. (1944), *Arch. Path.*, **37**, 294; Handler, S. L. (1945), *Amer. J. clin. Path.*, **15**, 107) for 7-15 minutes, the intensity of the stain being checked by the use of the microscope. Following rapid differentiation in 0.2 p.c. acetic acid and rapid dehydration in 95 p.c. and absolute alcohol the sections are cleared in xylol and mounted. If staining is satisfactory inclusion bodies should stain crimson, nuclei purple and the cytoplasm of the bladder and trachea epithelial cells grayish-purple; red blood cells stain orange; collagen stains blue-green and muscle red.

G. M. F.

Rapid Celloidin Impregnation of Bone.—J. J. BERTRAND ("A Rapid Method for Celloidin Impregnation of Undecalcified Bone," *Science*, **107**, 152). When working with Au¹⁹⁸ which has a half-life of 2.7 days difficulty was experienced in obtaining autoradiographs of bone because of its rapid rate of decay. Routine celloidin embedding of bone (undecalcified) requires 7-10 days. The following method was therefore adopted. The bone specimen is placed in the embedding dish and enclosed in a metal apparatus with a close-fitting lid which can be screwed down. A vacuum of 5 mm. Hg. was obtained with a Hivæ air pump and continued for 60 minutes. Celloidin is then introduced by means of the negative pressure flowing through the central inlet and into the dish. Pressure was then applied (15 lb.) overnight. Satisfactory embedding is thus secured and bone sections can be cut at 4 μ .

G. M. F.

Isopropyl Alcohol and Paraffin Infiltration.—E. K. DOXTADER ("Isopropyl Alcohol in the Paraffin Infiltration Technic," *Stain Technol.*, 1948, **23**, 1-2, 4 refs.). Formalin-fixed tissues are washed overnight to remove the fixative. They are dehydrated, infiltrated, and embedded as follows:—Isopropyl alcohol : 60 p.c., 1 hour ; 99 p.c., 1 hour ; 99 p.c., 2 hours ; 99 p.c., (40-45° C.) 3 hours.

Melted tissue-mat : MP, 50-52° C., 1 hour ; MP, 50-52° C., overnight. Embed and block in tissue-mat of melting-point to suit cutting conditions. G. M. F.

The Diagnosis of Cancerous and Normal Tissues.—G. I. ROSKIN and M. E. STRUVE ("Differential Cytophysiological Diagnosis of Cancerous and Normal Tissues," *Stain Technol.*, **22**, 83-6, 13 refs.). A method for the differential diagnosis of cancer cells is proposed using methylene blue decolorized with sodium thiosulphate (hypo-sulphite methylene blue HLM) : this is prepared by dissolving 800 mg. sodium thio-sulphate in 10 ml. of 0.1 p.c. aqueous methylene blue and adding 3-5 drops of dilute hydrochloric acid (1 : 3). Frozen sections are treated with this reagent for 2-3 minutes, rinsed with a large amount of distilled water, then stained for 2-3 minutes with 0.05 p.c. aqueous acid fuchsin. Staining must be performed in a darkened room. According to the Russian workers the nuclei of normal cells stain dark blue while the nuclear structures are quite distinct the nuclei of cancerous cells either remain almost colourless, assume a diffuse very light blue tint or a slightly perceptible pale pink colour. The nucleoli of malignant cells are more intensely coloured than the almost colourless nuclei, although their blue tint is far less intense than that of normal nuclei. The protoplasm of normal and malignant cells is stained red by fuchsin S. It is suggested that this method, combined with measurement of the nucleoli which are larger in cancer cells, yields reliable results. G. M. F.

The Prevention and Treatment of Fungus Infection of Negatives.—B. S. CRANDALL ("Removal and Prevention of Fungus Stain of Photographic Negatives in the tropics," *J. biol. phot. Ass.*, 1947, **16**, No. 2, 78-80). In warm moist climates photographic negatives are often rendered useless because they become covered with fungus mycelium. For 35 mm. film negatives the problem can be solved by storing over silica gel in tins which can be hermetically sealed. For larger negatives this method is impracticable. In the Amazon region immersion in the standard fixing-bath hypo for 5 or 10 minutes cleared the stain from the negative and acted as a fungicide. Negatives so treated remained free from fresh infection for four months. Negatives have been protected from fungus infection for ten months by immersion in a 1 in 1000 solution of mercuric chloride immediately after washing or in a solution of "lignasan" containing 2.4 g. per litre. [Lignasan is ethyl mercuric chloride.] G. M. F.

The Use of Immersion Oil.—A. PILPER ("A New Way of Applying Immersion Oil," *J. Path. Bact.*, 1947, **59**, 486). Immersion oil is usually applied by immersing a glass rod in a bottle of the oil. This is unsatisfactory, especially if the microscope is being used in a nearly horizontal position. A dropping bottle, known as a Schuster bottle, is preferable : its volume is about 30 ml. but it works best with only a few ml. of oil. The oil is poured in through an opening which is closed by a cork. G. M. F.

Embedding Eyes.—M. E. CARSTEN ("A Rapid Procedure for the Embedding of Eyes," *Arch. Path.*, 1947, **44**, 96-100, 3 refs.). Eyes can be rapidly embedded with very little shrinkage or distortion by means of water-soluble "carbowax" (polyethylene glycol) and "carbowax" ether (prepared by the Carbide and Chemicals Corporation and the Mellon Institute, U.S.A.). The eye is first fixed in Bouin's solution for two or

three days : cut in half and transferred to a mixture of carbowax 1000 and distilled water, equal parts, for 30 minutes at 37° C. Then it is transferred for 2 hours into four successive changes at 37° C. It is next placed in a mixture of "carbowax 1000" and "carbowax 1540 distearate" equal parts for 30 minutes and into four successive changes of carbowax 1540 distearate for 2 hours at 45° C. During the last change it remains overnight and is embedded in fresh carbowax in the morning. The entire process of embedding, sectioning, and staining takes 4 days.

G. M. F.

Staining Leprosy Bacilli.—G. L. FITE, P. J. CAMBRE, and M. H. TURNER ("Procedure for Demonstrating Lepra Bacilli in Paraffin Sections," *Arch. Path.*, 1947, **43**, 624). *Mycobacterium leprae* is not so acid fast as *Myco. tuberculosis* and is thus more difficult to stain in paraffin sections. The following technique gives good results irrespective of the techniques of fixation and embedding. Paraffin is removed in two changes (1–2 minutes each) of 1 part of oil (cotton seed, olive or ground nut) in 2 parts of xylene. The excess oil is drained off, wiped and blotted. If mercury crystals are present the sections are treated with strong iodine solution (2 minutes) followed by hyposulphite or thiosulphate solution. The sections are then washed in tap-water : stained in any standard carbol fuchsin in the cold for 15–30 minutes and again washed in tap-water. Decolourization for 1–2 minutes is carried out with 1 p.c. concentrated hydrochloric acid in 70 p.c. alcohol till the slides are a faint pink colour. After washing sections are counterstained with Loeffler's alkaline methylene blue for 30 seconds, washed, allowed to dry, and mounted in any synthetic mounting medium.

G. M. F.

Photo-electric Counting.—C. LAGERCRANTZ ("Photo-electric counting of Individual Microscopic Plant and Animal Cells," *Nature*, 1948, **161**, 25–26). An optical system is described by means of which red blood cells, yeast cells, and other microscopic cells can be counted. The cells must be uniformly distributed and the cell suspension free from contamination with foreign particles that can be recorded by the electronic system.

G. M. F.

Decalcifying Bone.—I. M. RICHMAN, M. GELFAND, and J. M. HILL ("A Method of Decalcifying Bone for Histologic Section," *Arch. Path.*, 1947, **44**, 92–95, 11 refs., 2 figs.). This method employs the forced migration of positively charged calcium to a negatively charged electrode or a "deplating" of the calcium carbonate and phosphate from the bone. The bone is placed in a container of plastic with holes and a locking lid. Multiple holes in the container allow free fluid interchange round the bone. The platinum wire electrode impinges on and holds fast the bone specimen. Good results are obtained with a 6-volt storage battery. A constant source of current is produced by the use of a rectifier attached to the regulation 110 A.C. circuit. With the use of a rheostat the amperage may be controlled to produce desirable results. The vessel containing the electrolyte bath is of glass and the bath should be kept at 30–45° C. The electrolyte bath contains 8 p.c. hydrochloric acid and 10 p.c. formic acid as the electrolytic medium. Two to six hours is sufficient to produce proper decalcification.

G. M. F.

A' Giemsa-type Stain.—A. BAHRAMI ("Un colorant de remplacement du Giemsa," *Bull. Soc. Path. exot.*, 1947, **40**, 110). If Giemsa stain is unobtainable, good results can be obtained with a "mercurial blue" and eosin mercurio chloride, 1 g., is dissolved in 150 ml. of distilled water : a 10 p.c. solution of sodium hydroxide is added to obtain a precipitate of yellow oxide of mercury. The precipitate is filtered off and washed with distilled water till the washings are neutral to litmus paper. To the

precipitated mercury 110 ml. of a 1 p.c. aqueous solution of blue are added and the mixture autoclaved for one hour at 120° C. The liquid thus obtained is of a violet-blue colour which if too violet is rejected. The liquid is filtered and evaporated to dryness in a water bath. To prepare the stain 1 g. of the violet-blue powder and 0.5 g. of eosin are dissolved in a mixture of 50 ml. of absolute methyl alcohol and 50 ml. of neutral glycerine. The stain, which may be used after 24 hours, is used in the same way as Giemsa.

G. M. F.

Color-translation.—E. H. LAND (*Chemical and Engineering News*, 1948, **26** (18), 1280 (May 3)). Tissue is photographed with ultra-violet light of three different wavelengths selected to give the desired contrast. The three photographs are then combined by printing each in a visible primary colour to give a picture in which the colours are translatable in terms of ultra-violet absorption. Actually, the colours obtained bear no relation to any colours in the specimen, which may appear quite colourless in visible light. Colour-translation photography requires special equipment, including a reflection microscope in which curved mirrors are employed as lenses. This and other equipment and materials are being developed by the Polaroid research group. Cell components are easily discernible and in several cases cancer tissue is prominently differentiated from normal tissue. This emphasizes its chemical difference from normal tissue, since ultra-violet absorption is linked to chemical composition.

CYTOLOGY.

Direct Examination of Bacteriophage.—A. W. HOFER, and O. W. RICHARDS ("Observation of a bacteriophage through a light microscope," *Science*, 1945, **101**, 466-8). Bacteriophage preparations treated with auramin and examined under the microscope whilst irradiated with ultra-violet light from an H4 mercury arc showed bright yellow particles in a dark field. Further it was found that in the presence of pea nodule organisms methylene blue or rose bengal, whilst tinting the organism slightly, stained the bacteriophage heavily. Examination was rendered easier by the use of phase contrast. By these methods the apparent sequence of lysis was determined as (i) the bacteriophage becomes attached by a slender fibril to the bacterium; (ii) the bacteriophage increases in size and the fibril shortens; and (iii) the cells break up leaving faintly staining cell remnants among which are numerous minute brilliantly coloured particles. Experiments with two different strains of pea-root nodule organisms and two bacteriophages specific to them, indicated that the process is not well defined but consists of a number of variable phenomena. Points noted were loss of motility of cells within 1-2 minutes; clumping of cells; appearance of a blue coloration in cells; tendency of cells to assume vertical instead of horizontal position; uniting of bacteriophage with cell and eventual break up of cell as above; appearance of relatively large refractile bodies in cell; appearance of masses of protoplasm containing highly refractile bodies in the medium; very large cells of grotesque shape and apparent large masses of cell protoplasm containing small blue bodies arranged to suggest chromatin like material. In no instance did all these phenomena occur in any one suspension. Evidence from electron microscope studies, stained preparations, and phase contrast for the process of lysis is presented and appears to be consistent. G. C.

Induced Bacterial Mutations.—M. DEMEREC, and R. LATARÉT ("Mutations in Bacteria Induced by Radiations," *Cold Spring Harbor Symposia on Quantitative Biology*, **11**, 38-50). Direct genetic tests for mutations in bacteria being impossible, the authors

have tested for specific mutations for resistance to certain bacteriophage. Two separate lines—B and B/r—of the B strain of *Escherichia coli* were used in conjunction with bacteriophages T1 and T2. Radiations used were from a G.E.C. germicidal lamp with c. 80 p.c. output of wave band 2537 Å and an X-ray machine with two tubes with tungsten anti-cathodes, face to face, 30 cms. apart and operating at 180 kV. and 25 mA. Filtering was only by the glass of the tubes. Bacteria were grown and irradiated in a synthetic ammonium chloride-glucose-phosphate buffer medium. Bacterial suspensions 1 mm. deep were irradiated in open Petri dishes.

Assays were done by plating samples of diluted culture on to broth agar plates and counting colonies after 24 hours incubation at 37° C. For estimating mutants already present the agar plates were previously coated with c. 10^8 particles of phage. For delayed mutants the plates were sprayed with phage after a period adequate for the required number of divisions. In both cases all sensitive bacteria were eliminated by the phage whilst resistant forms survived and formed colonies which were later counted. For full experimental details and methods of dosage estimation the original paper should be consulted.

Mutations were obtained in both strains. Figures indicate that for U.V. there is a rapid increase in mutation rate up to a dosage of 4000 ergs per mm.² and then a gradual falling off. With X-rays the "killing" rate was so high that comparable figures were not obtainable, but it appears that the relation between zero point mutants and dosage follows a straight line. Comparison of the effects of U.V. on growing and resting bacteria showed little difference. On a quantitative basis X-rays were found to be more efficient than U.V. in producing mutations. It is concluded that there is no relationship between the ability of a radiation to produce sterilization and its ability to produce mutations. With comparable amounts of energy absorbed by the cell the mutation rates from U.V. and X-rays are similar, but the sterilization rate is higher with X-rays than with U.V.

G. C.

Sex Hormones and Chromosome Size.—J. J. BIESELE, and G. GASIC ("Sex Hormone Effects on Chromosome Size in Leukæmic and Normal Lymphocytes of C. 58 Mice," *Cancer Research*, 1947, 7, 65-9). On the basis of the observation that metaphasic chromosomes of the cells of lymphatic leukæmia were usually larger in male than female hosts investigations of chromosome size in leukæmic and normal lymphocytes as influenced by various sex hormones were carried out. Groups of 1 month old C. 58 mice were used as follows:—Normal males; Normal males implanted with a 10-13 mg. pellet of testosterone propionate and cholesterol (2:3); Normal females; Intact females implanted as before; Spayed females; Spayed females implanted as before; Spayed females implanted with a 6-7 mg. pellet of testosterone propionate. Pellets were implanted subcutaneously in the interscapular region at the time of spaying; a second pellet being inserted in the inguinal region after 30 days in the mice receiving pure testosterone propionate. Intraperitoneal inoculations of leukæmic cells suspended in saline were done at intervals between 21 and 60 days after treatment. Animals then survived approximately 6 days; those containing pellets living a few hours longer than the untreated. An inguinal node from each newly dead mouse was fixed in alcohol-acetic acid; a portion was stained in aceto-carmin and smeared out. Measurements of chromosomes were done under oil immersion with green light.

Results showed that chromosomes decreased in size through the series: males, intact females, spayed females. Testosterone propionate tended to maintain leukæmic chromosome sizes in spayed females at the size found in males. Chromosomes of normal lymphocytes were smaller than usual in the animals receiving androgen. Ovariectomy alone did not alter the size of normal lymphocyte chromosomes.

G. C.

Conifers Breeding and Grafting.—M. C. L. KEILANDER ("Breeding and Grafting of Conifers," Summary of an article in the *Svensk Pappersmasse Tidning: The Anglo-Swedish Rev.*, April, 1947, 63–66, 8 figs.). The breeding of forest trees from so-called *élite* trees of "plus" variants is described in which they are surrounded with scaffolding to facilitate pollination and grafting experiments. Over 12,000 individual trees of *Pinus aibus* and *Pinus sylvestris* have been registered from Skåne to northernmost Noorland. Careful differentiation is made between individuals showing good appearance and form owing to environmental conditions and those exhibiting good hereditary qualities, and only when positive results are obtained is work started on the genotypically best trees. Experiments are in progress in the production of polyploidy by chemical agents. A number of tetraploid Spruces with forty-eight chromosomes instead of the normal twenty-four have been produced, and in Pine and Larch there are several plants which most probably have an increased chromosome number: giant races are envisaged from these beginnings. Freezing experiments are carried out to ascertain winter hardiness of non-indigenous varieties. F. C. G.

Chromosomes in Evolution.—C. W. METZ ("Duplication of Chromosome Parts as a Factor in Evolution," *The Amer. Nat.*, 1947, **81**, 81–103, 4 figs.). The author considers the primary task in the discussion to "get some conception of how new materials have been added to the chromosomes during the course of evolution" and describes the new unit to be of proper size, self-reproducing, able to correlate its rate of growth and reproduction with other genic units and able to fit in function and in harmony with its compatriots; he states that the duplication of chromosome parts has probably been one of the most important factors in evolution. Discussion follows on nature and type of segment duplication in *Drosophila* salivary gland chromosomes and the significance of the heterozygous condition in this respect.

The theory of the additive genetical effects attributed to multiple band repeats which may be the cause of orthogenesis involving especially rapid evolution is dealt with and contributions to this study are found in the researches of McClintock on *maize* where the loss of one to two of adjacent very short segments near the tip of chromosome-9 leads to chlorophyll deficiency. Previously unpublished conclusions of the latter worker are included that the tip of chromosome-9 is made up of a series of identical genes, each contributing specific quantity of substance necessary for chlorophyll production. The phenomena of "triple repeat" synapsis in *Sciara* is discussed in detail and illustrated with an excellent photomicrograph. F. C. G.

Electron Microscopy of Chromosomes.—J. T. BUCHHOLTZ ("Methods in the Preparation of Chromosomes and Other Parts of Cells for Examination with an Electron Microscope," *Amer. J. Bot.*, 1947, **34**, 445–54, 22 figs., plus 18 refs.). The methods employed in preparation of biological material for examination under the electron microscope are described including the cutting of sections with the high-speed rotary microtome. Observations are carried out on pollen mother-cells of *Maize* and eggs of *Tsuga canadensis*. With the latter small quantities of cytoplasm were extracted with a micro-pipette and treated as a suspension similar to the methods employed for bacteria by diluting with distilled water. With the maize material entire anthers were fixed in Carnoy 3:1 and stained in bulk with Mallory's Hæmatoxylin, the P.M.Cs. were dissected out in glycerine solution the consistency of syrup. The stage of loose open pachetene was favoured and cells were dissected partly by hand and partly with a Zeiss microdissection apparatus. Isolated fragments of chromonemata were obtained, the glycerine dispersed with absolute alcohol followed by amyl acetate and then colloidion dissolved in the same solvent of about 2 p.c. concentration. The resultant film, which should be as thin as possible, is floated out on a water surface and transferred to

the grid. The centre of the grid has a small enlargement made with a fine sewing needle over which the object is orientated with the compound microscope, this serves as an aid to the location of the object under the electron microscope. With this method the object is embedded in the film so the shadowing technique cannot be employed. Four electron micrographs are included showing the results of this part of the work and appear to show a slender thread to which are attached a considerable number of granules of a variety of shapes and sizes. A certain amount of coiling is apparent in the thread. The writer contends that the findings of light microscopy of the past fifty years have not been contradicted in important details.

A variety of bodies presumed to be mitochondria were found in abundance which possess a thin membrane and internal structures designated as "alveoli"—numerous transparent spheres—and "kernels" which appear as small dense rounded areas, in some cases these structures appear to form patterns in their disposition. Some of these "mitochondria" appear as branched forms and others hanging together in chains, they appear to divide transversely to the long axis as do bacterial cells. The similarity to bacteria is discussed in which it is considered that mitochondria may be analogous to bacteria but not homologous.

F. C. G.

Transfer of Desoxyribose Nucleic Acid.—A. H. SPARROW and MARIAN R. HAMMOND ("Cytological Evidence for the Transfer of Desoxyribose Nucleic Acid from Nucleus to Cytoplasm in certain Plants," *Amer. J. Bot.*, 1947, **34**, 739-45, 15 refs., 12 figs.). Evidence is advanced that Feulgen positive bodies arising from an unknown point of origin in pollen mother-cells of *Lilium* sp., *Tpillum* sp., and a few additional types, are present within the nucleus, attached to the nucleus by a very fine stalk or lying free in the cytoplasm. In a few instances they seemed to be attached to the chromosomes and in other cases were attached by a fine strand to the nucleus of an adjacent cell (*Cytomixis*). In cells with a high number of Feulgen positive bodies, the nucleus frequently stained very lightly and suggests that the chromosomes have given up their nucleic acid charge. Conclusive proof is lacking that fusion takes place between the bodies which vary in size between 0.3μ to 7μ and number from one to sixty per cell. They show a high absorption at $2437 \text{ \AA}$ and $22650 \text{ \AA}$ which is characteristic of the purine and pyrimidine bases of nucleic acids (or nucleotides) and this, coupled with the positive Feulgen reaction, the authors claim is convincing evidence for the presence of Desoxyribose nucleic acid in the Cytoplasmic bodies. The absorption at $2804 \text{ \AA}$ probably indicates the presence of protein. The carmine-fast green technique gives evidence that cytoplasmic bodies are not extruded nucleoli and probably do not contain nucleolar substance. The interpretation of the phenomena is laid out as follows: (i) caused by abnormal environmental conditions; (ii) formed in damaged or diseased cells; and (iii) found in healthy cells under certain limited conditions and that in such conditions they play a useful role in cellular metabolism. The authors do not consider virus infection a cause.

F. C. G.

Sieve-tube Inclusions.—KATHERINE ESAU ("A Study of Some Sieve-tube Inclusions," *Amer. J. Bot.*, 1947, **34**, 224-33, 13 refs., 55 figs.). The history of the Protoplast in the development of the sieve-tubes of *Vitis*, *Rubus*, *Gossypium*, and *Eucalyptus* is described with special reference to the inclusions. Nucleoli are shown to be extruded into the cytoplasm, assume a definite form and remain morphologically unchanged so long as the sieve-tube element exists as an intact cell. Nucleolar extrusions, however, do not always occur, but are absent in some plants while present in others. In *Rubus* nucleolar material is extruded from the nucleus before disintegration of the latter, usually the larger nucleoli are ejected before the smaller.

The other inclusions dealt with are the "slime bodies," which are described as discrete structures of colloidal substance that occurs in the protoplasts of young sieve-tubes in many dicotyledons, are of higher density than the cytoplasm, semi-fluid and greatly variable in shape. They are interpreted as proteinaceous bodies: with the differentiation of the sieve tube element they spread out, lose density and fuse together and appear to lose their identity within the other cellular contents. In killed material they usually accumulate near a sieve plate and form the so-called "slime plug."

In *Vitis vinifera* slime bodies also occur in the companion cells. The presence of slime bodies presents a stage in the differentiation of the contents of the sieve-tube element. It is contended that, to identify the extruded nucleoli with slime bodies is an error, as the two kinds of structure can be readily differentiated. The technique is as follows: fixed in Craft III (Sass, 1940, "Elements of Bot. Micro-technique," p. 19), cut 10 μ , stained Heidenhain's Iron alum-haematoxylin and counterstained with orange G. Some *Vitis* material was not embedded but cut on a sliding microtome, stained with Bismarck brown and iodine-green and mounted in dark Karo syrup; some was cut fresh and treated with iodine and aniline blue and mounted in glycerine.

F. C. G.

PROTOZOA.

In Vitro Development of Avian *Plasmodium*.—W. TRAGER ("The Development of the Malaria Parasite *Plasmodium lophuræ* in Red Blood Cell Suspensions," *J. Parasitol.*, 1947, **33** (4), 345-50). The author describes a method of maintaining *Plasmodium lophuræ* in a suspension of the host's erythrocytes, using the following method. First, media are prepared which represent balanced solutions of high potassium content; then a red cell extract is prepared, and a suspension of parasitized erythrocytes. The latter was made by withdrawing from the vein of a normal duck 23 ml. of blood and mixing it with 2 ml. heparin solution; then the blood was centrifuged and 10-11 ml. of the supernatant plasma was drawn off, after which the cells were re-suspended in the remaining plasma. Five ml. of blood were then drawn from the vein of an infected duck into 0.5 ml. heparin solution. The parasitized blood was mixed with the concentrated normal blood in the proportion of 1 ml. of the former to 4 ml. of the latter. Cultures were made in 50 ml. Erlenmeyer flasks containing 4.5 ml. of the medium, to each of which was added 1.5 ml. of parasitized red blood cell suspension, giving concentrations of about 50,000 parasites and 1,000,000 red cells per cub. mm. Each flask was provided with a rubber stopper carrying gas inlet and outlet tubes, after which the flasks were placed on a rocker in an incubator at about 40° C. and were connected to a source of a mixture of 95 p.c. air and 5 p.c. CO₂.

Observations were carried out by removing a drop of material from the flask and making a film stained with Giemsa's solution. By this method multiplication of 2-3 generations of *P. lophuræ* was obtained within the erythrocytes in the course of 2 days; on subcultivation they continue to develop for 2 days longer. During a period of 8 days, with subcultures made every 2 days, the parasites produced 170 generations.

C. A. H.

Fibrils in *Opalina*.—W. B. COSGROVE ("Fibrillar Structure in *Opalina obtrigonoidea* Metcalf," *J. Parasitol.*, 1947, **33**, 351-7, 1 pl.). Description of the superficial fibrillar structures in the ciliate *Opalina obtrigonoidea* from the rectum of the frog *Rana pipiens*. The rectal contents with the ciliates were fixed in an excess of one of the following fixatives: Bouin's, Hollande's, Schaudinn's, Zenker's, Helly's, Flemming's, Champy's, 2 p.c. osmic acid and saturated corrosive sublimate. Washing,

dehydration and clearing were carried out in bulk in centrifuge tubes, with the addition of eosin to the absolute alcohol. Cleared specimens were embedded individually in paraffin wax after remaining ten minutes in the oven, and thin sections were cut of small blocks, each containing a single ciliate. The best results were obtained after staining with Mallory's triple stain and iron hæmatoxylin differentiated with 10 p.c. aqueous hydrogen peroxide. The Opalinid ciliates treated by these methods revealed complete ciliary rows extending spirally around the body, as well as intercalated rows. A pair of fibrils running along the anterior end below the pellicle give rise to the ciliary rows and to stout cilia. It is thought that they are homologous to the neuromotor apparatus. Each basal granule of a row is connected with those in the adjacent rows by oblique fibrils. Stout fibrils extend from some of the basal granules through the endoplasm and are attached either to others on the opposite side or to the nuclear membrane. This study has produced no evidence of the affinities between the Opalinida and the Plasmodroma, but point to their close relationship with the Ciliata.

C. A. H.

HELMINTHS.

Morphology of the Cysticeroid of the Fowl Tapeworm *Raillietina cesticillus* (Molin).—C. L. WISSEMAN (Trans. Amer. Micro. Soc., 64 (2), 145–50). Additional information is provided on the morphology of the cysticeroid of *Raillietina cesticillus*. A wide range of developmental stages was obtained by examination of ground-beetles (*Anisotarsus agilis*) which had been experimentally infected from gravid proglottids of the adult tapeworm. The gross morphological changes in successive phases from onchosphere to invaginated cysticeroid are described which support and supplement studies made by other workers.

O. D. S.

New Trematodes from Puget Sound Fishes.—S. P. ACENA (Trans. Amer. Micro. Soc., 1947, 66 (2), 127–39). Trematodes found in the alimentary canal of the fishes *Aspicottus bison*, *Sebastes nebulosus*, and *Parophrys retulus* are described. Two new species of the genus *Lepidapedon* Stafford 1904 are described and the names *L. pugetensis* and *L. calli* are proposed.

A new genus of trematode of the sub-family *Derogenetinae* is described from the stomach of *Aspicottus bison* Girard, and the name *Intuscirrus aspicotti* n. gen. et n. sp. is proposed. It is suggested that *Lepidora gadi* Yamaguti (1934) should be included in the genus *Lepidapedon*.

O. D. S.

***Neodiplostomum banghami*, a New Diplostomatid Strigeoidean Trematode from an Eagle.**—F. W. PENROD (Ibid., 144–48). Ten worms taken from an immature Bald Eagle, *Haliaeetus leucocephalus* (Linné, 1766) were used for the purpose of this investigation. A new species is created and the name *Neodiplostomum banghami* is proposed.

O. D. S.

Preliminary Survey of the Helminth Parasites of Muskrats in Northwestern Oregon, with description of *Hymenolepis ondatrae*.—C. L. RIDER and R. W. MACEY (Ibid., 176–81). A preliminary survey of the helminth parasites of Muskrats in this area was undertaken in view of the economic importance of the host. Thirty-four animals only were examined but the presence of *Echinostoma revolutum*, *Notocotylus urbanensis*, *Hymenolepis ondatrae* n. sp. in 35 p.c., *Tænia tæniæformis* in 29 p.c. and larvæ of a nematode believed to be *Trichostrongylus* in 9 p.c. indicate that these parasites are probably quite common.

A description of *H. ondatrae* as a new species is included.

O. D. S.

Two New Trematodes from Philippine Fishes.—R. F. ANNÉREAUX (*Ibid.*, 172–75). Two new species are created. *Hexangium secundum* n. sp. is described from one adult specimen recovered from the intestine of *Teuthis concatenata* (Cuvier and Valenciennes) and *Hysterolecitha acanthuri* n. sp. from two complete and one fragmented specimen recovered from the stomach (probably) of *Acanthurus triostegus* (Linn.).

O. D. S.

The Genus *Anonchotænia* (Cestoda : Dilepididæ) from North American Birds, with the Description of a New Species.—R. RAUSCH and B. B. MORGAN (*Ibid.*, 203–11). A brief review is given of the literature relating to the genus *Anonchotænia* with special reference to North American forms. A new species, *A. quiscali* is described from the Common Grackle, *Quiscalus versicolor* Vieill.

O. D. S.

FORAMINIFERA.

Asterigerina.—A. TEN. DAM ("Structure of Asterigerina and a New Species," *Jour. Pal.*, 1947, **21**, No. 6, 584–6, 6 text-figs.), November. Species of Asterigerina are studied including one new species, from the Middle Oligocene, the Middle Eocene, and the Middle Miocene. The perfect preservation of the new species *A. lutetiana* allows minute study of the fundamental details of internal structure including the inner structure of the chambers. The generic apertural characters are well shown and the final chamber is characterized by its crescent-shaped apertural nature. The apertural slit is almost parallel to the periphery (but in other species where the stellar chambers do not reach the periphery it is more radial). The stellar chambers are not secondary since they distinctly alternate with the dorsal chambers, communication between the dorsal chambers being by way of the stellar chamber.

The two other species reveal the same fundamental structure as the new species.

G. H.

Bulimina and other Genera.—J. A. CUSHMAN and F. L. PARKER ("Bulimina and Related Foraminiferal Genera," *U.S. Geol. Surv.*, 1947, Prof. Paper 210-D, 55–176, pls. 15–31). Three subfamilies of the Buliminidæ are described including the genera Terebralina, Turrilina, Buliminella, Buliminoides, Ungulatella, Robertina, Pseudobulimina, Bulimina, Desobulimina, Neobulimina, and Globobulimina. A large number of species assigned originally to Bulimina have been assigned to other genera. Four of the species are new and one new name is proposed. Entosolenia is omitted because of the difficulty of placing many of the species. In many cases the description is based on actual material. The descriptions of all genera, species, and varieties are very full.

G. H.

A New Archaias.—G. A. DE NEVE ("A New Archaias Species from East Borneo," *Bull. Bur. Mines Geol. Surv.*, 1947, Indonesia, **1**, No. 1, 13–16, 4 text-figs.). Samples were collected from the Poelobang beds of East Borneo which yielded four known species of foraminifera and one new species—*Archaias vandervlerki*. This was determined as *Orbiculina* cf. *adunca* Fichtel and Moll by Rutten from the Tertiary of Java. A list of other species found with this species is given. The age of these beds is determined as Older Miocene by molluscs. The new species began its development in the Lower Miocene but its exact vertical range is not yet determined.

G. H.

Eocene Sediments in Hungary.—M. VAŠIČEK ("Remarks on the Microbiostratigraphy of the Magura Flysch in Moravia," *Věst. Stát. Geol., Republic Československé*, 1947, **22**, 250–6, 3 pls.). The many peculiarities of habit in foraminifera are discussed in relation to the Eocene sediments of the Moravian Magura Flysch. The sediments were subject to disturbances and the reactions of foraminifera to these are given.

The zones set up by the presence of foraminifera in the Cretaceous and Eocene are given. A table shows the faunal position in the various zones.

The new forms described include ten new species and one new genus *Remesella* with gaudryina-like test and chambers with incomplete secondary transversal septa.

G. H.

The Genera *Pulvinulinella*, *Parrella* and *Alabamina*.—J. A. CUSHMAN ("Some Notes on the Genera *Pulvinulinella*, *Parrella*, and *Alabamina*," *Contrib. Cush. Lab. Foram. Res.*, 1948, **24**, Pt. 1, No. 306, 13–14). These three genera have been considerably confused and after detailed study of the species and the generic definition laid down *Pulvinulinella* and *Alabamina* strongly suggest that they are intermediate stages towards the aperture of the Cassidulina group and should be placed in that family. *Parrella* on the other hand, with its apertural features, may be placed in the *Rotaliidae*, until ancestral species are studied in detail.

G. H.

Mississippi Foraminifera.—J. A. CUSHMAN and RUTH TODD ("Foraminifera from the Red Bluff-Yazoo Section at Red Bluff Mississippi," *Contrib. Cush. Lab. Foram. Res.*, 1948, **24**, Pt. 1, No. 304, 1–2). Abundant smaller foraminifera are found, well preserved in samples at close intervals from the Red Bluff-Yazoo section. These give representatives of the Upper Eocene and the Lower Oligocene. Nine new species are described and figured. A chart shows the distribution of the species. Lists are given of species restricted to the Red Bluff clay and the Yazoo clay respectively.

G. H.

New Genus from Cuba.—J. A. CUSHMAN and P. J. BERMUDEZ ("Colomia, a new genus from the Upper Cretaceous of Cuba," *Contrib. Cush. Lab. Foram. Res.*, 1948, **24**, Pt. 1, No. 305, 12, 13, pl. 2). This new genus and species from the Habana formation of Cuba occurs with *Globotruncana*, *Gumbelina*, *Ventilabrella*, *Eouvigerina* and others. It apparently bears relationship with the recent *Ungulatella* of the *Buliminidae*. *Colomia* has a conical test with its earliest chambers indistinct and later ones uniserial. It is circular in transverse section, with vertical columns connecting walls of the adjacent chambers; the wall is calcareous and perforate; the aperture is a slightly arcuate narrow opening in the middle of the apertural face.

G. H.

New Foraminifera from Trinidad.—J. A. CUSHMAN and P. BRONNIMANN ("Some New Genera and Species of Foraminifera from Brackish Water of Trinidad," *Contrib. Cush. Lab. Foram. Res.*, 1948, **24**, Pt. 1, No. 307, 15–21, pls. 3, 4). Three new genera and thirteen new species are described from inshore mud in Mangrove swamps of river estuaries of the west coast of Trinidad. Most samples are from the coastal area between the Caroni and Godineau rivers. The fauna is unusual and an ecological study is to be made. *Ammonostruta*, a new genus of the family Lituolidae has close coiled planispiral test in the early portion, uncoiling early and in the adult elongate curved chamber each forming more than half the periphery of the oval test; the wall is arenaceous with the aperture as a series of pores on the curved lower end of

the last-formed chamber. *Trochamminita*, a new genus of the family Trochamminidae, resembles *Trochammina* in the early stages, with the chambers added in an irregular manner later, the aperture consisting of a rounded opening in the chamber wall with a slightly raised border. *Criboelphidium*, a new genus of the family Nonionidae, resembles Elphidium, but with the apertural face with rounded openings, giving supplementary apertures. G. H.

The Genus *Trocholina*.—F. R. S. HENSON ("Foraminifera of the Genus *Trocholina* in the Middle East," *Ann. Mag. Nat. Hist.*, 1947, Ser. 11, 14, No. 115, 1948, 445-59, pls. 11-13). Species of *Trocholina* are some of the most common of the foraminifera from the Jurassic and Cretaceous of the Middle East. These are determined after careful comparison with Paalzow's figures and an axial section of *Trocholina* (Involutina) conica (Schlumberger) from Schlumberger's collections. On this basis a reinterpretation of the genus is given. A full comparative description is given of *Trocholina* and the author summarizes these. The nucleocoenoch of *Trocholina* is like that of *Cornuspira*, the wall of the spire is calcareous, but not fibrous, microgranular, sometimes perforate, probably originally chitinous, with the chitin lost in the process of fossilization as is usual. Although the granules on the ventral surface suggest umbilical pillars, these are absent and the umbilicus filled with a coarsely granular calcite plug. In the extreme, compressed variants of *T. lenticularis* Henson sp. n.v., the whorls become planispiral and clearly cornuspirine. The range of *Trocholina* is given as early Mesozoic, possibly Permian to Cenomanian in the Middle East and most common in the Middle-Upper Jurassic and Middle Cretaceous. It is widely distributed and often abundant. Four new species and one new variety are described. G. H.

Lagenid Foraminifera.—W. J. PARR ("The Lagenid Foraminifera and Their Relationships," *Proc. Roy. Soc. Vict.*, 1947, 58, Pts. 1-11 (N.S.), 116-30, pls. 6, 7). The opinions currently held as to the position of the lagenid foraminifera is reviewed and evidence is submitted to show the relationship between *Lagena*, *Oolina* (with *Entosolenia* as a synonym), *Fissurina* and the hooded forms usually referred to *Ellipsolagena*, which he regards as identical with *Fissurina*, the latter having preference. The conclusions are that the group are not end forms and belong to the same family, not a polyphyletic group of end forms derived from multilocular genera belonging to several families. The family Lagenidae is proposed for the unilocular forms with the *Nodosariidae* restricted to the multi-locular forms. A new genus *Parafissurina* is erected to accommodate the hooded forms which have usually been referred to an *Ellipsolagena*. The earliest lagenid forms are from the Jurassic—only rarely—including *Lagena* and *Oolina* and in the lower Cretaceous they become more varied and *Fissurina* appears in the Upper Cretaceous. *Parafissurina* appears in the Eocene. All are present in the Tertiary and distribution is world-wide in recent seas.

The author's key to the family is that *Lagena* has a test with terminal aperture and with apertural neck and without entosolenian tube; *Fissurina* and *Oolina* may have apertural neck with entosolenian tube; *Oolina* is circular but in transverse section, *Fissurina* is compressed. *Parafissurina* has a test with subterminal aperture and entosolenian tube. G. H.

Foraminifera from the Paleocene.—J. A. CUSHMAN ("Some New Foraminifera from the Paleocene of the Southern United States," *Contr. Cush. Lab. Foram. Res.*, 1947, 23, Pt. 4, No. 301, 81-86, pls. 17, 18.) Ten new species and three new varieties are described from Alabama, Arkansas, and Texas. G. H.

A new Genus.—J. A. CUSHMAN and R. M. STAINFORTH "A New Genus and some New Species of Foraminifera from the Upper Eocene of Ecuador," *Contr. Cush. Lab. Foram. Res.*, 1947, **23**, Pt. 4, No. 300, 77–80, pl. 17). One new genus and eight new species are described from the Seca and Mateo formations of the Upper Eocene of Ecuador. The new genus *Buliminellita* of the family Buliminidæ is described. It differs from, and is evidently derived directly from, *Buliminella*; differing from the latter by a terminal aperture with a distinct neck in the adult stage. The genotype is *Buliminellita mirifica*, sp. nov. from the Seca formation at Punta Mambra, Ecuador. G. H.

New Species.—J. A. CUSHMAN ("New species and New Varieties of Foraminifera from off the South-eastern Coast of the United States," *Contr. Cush. Lab. Foram. Res.*, 1947, **23**, Pt. 4, No. 302, 86–92, pls. 18–20). Fourteen new species and three varieties are described and figured from the area from Cape Hatteras South to Southern Florida. Data is given with regard to temperatures, depths, and distribution of species. The depths go down to 120 metres. G. H.

Foraminifera from South Australia.—IRENE CRESPIN ("Foraminifera and other Micro-fossils from some of the Tertiary Deposits in the Vicinity of Ardinga Bay, South Australia," *Trans. Roy. S. Austr.*, 1947, **70**, Pt. 2, 297–301). The results of microscopic examination of collections from the glauconite deposits of material at Maslin Beach, Blanche Point and Ardinga Bay vicinity is given. The sediments are Middle Miocene referring to the Balcombian State. Abundance of certain forms suggests a correlation with the Longford Substage of the Balcombian. The assemblage of foraminifera and other microfauna is typical of the Balcombian Stage of the Middle Miocene of the Victorian Tertiaries. Some species of Foraminifera suggest reference to the Longford part of the Balcombian, vide Gippsland, and this is the conclusion reached as to the age of all the samples. Foraminifera give distinct evidence for Balcombian age although many species range from Middle Miocene to Recent. G. H.

INDUSTRIAL MICROSCOPY.

Baking.—S. H. CARLETON ("Baking under the Microscope," *Scientific American*, Nov., 1947, 216–18, 7 figs.). A method is described for micro-baking of various mixtures of cakes to ascertain the changes that take place during the process of cooking. The apparatus consists of a baking "pan" of a thin glass plate and the oven made up of a piece of thin insulating material through whose whole length a hole is drilled; a length of resistance wire serves as a heating element. The entire oven when complete is no more than half an inch in height.

A colour film is produced and records the movements of particles and the formation of air cells. For easy identification the shortening is coloured red with an oil-soluble dye which does not affect the colour of the water component. The flour is dyed purple with iodine. The changes observed taking place in the baking process are recorded and three photomicrographs are included showing the effects of variations in the composition of the batter. Polarized light revealed the critical stage of the solidification of the cake mixture by the behaviour of the starch grains. It is considered that much improvement is to be looked for in the baking practice on account of these researches. F. C. G.

Coal Petrology.—J. M. SCOFF (" Botanical Aspects of Coal Petrology : Coal from the Coos Bay Field in South-western Oregon," *Amer. J. Bot.*, 1947, **34**, 335–45, 22 photomicrographs and 8 refs.). The methods used in the preparation of coal sections are described and the various characteristics revealed such as varying degree of preservation of the woods and fungi and infiltration with waxy material etc. This waxy substance is not thought to be ordinary resin for in some cases fungal mycelia were found to have grown within it. Three different methods of preservation of the structural characteristics are recognized : (i) waxy material may impregnate the tissues and protect it from complete compression, (ii) impregnation by dispersed humic material but more rare, and (iii) the completely collapsed type of preservation with elimination of lumin cavities. In some cases humic material was found to be filling the lumens of tracheæ.

F. C. G.

The Revelations of the Microscope.—*Bull. Assoc. Tech. de l'Ind. Papetière*, 1948, **2** (3), 35–66). The steps in the paper-making process have been investigated microscopically, from the wood used as raw material up to the final chemical wood paper. Photo-micrographs are reproduced showing (1) a semi-hard cellulose fibre ; (2) a mechanical wood fibre ; (3) the effect of grinding ; (4) a crushed fibre ; (5) end of a crushed fibre ; (6) and (7) crushed fibres in polarized light ; (8) beaten fibres ; (9) fibre deformed into bands of fibrils ; (10) fibre split into bands of fibrils ; (11) ends of fibres deformed into strips ; (12) fibres and bundles tending to felt (taken in semi-polarized light) ; (13) bundles of fibrils and fibres tending to twist ; (14) twisting fibres ; (15) twisted fibres ; (16) bundles of fibres detaching themselves from the general texture ; (17) fibrils and bundles of fibrils felting ; (18) fibrils twisting ; (19) bundles of fibrils and inter-lacing fibrils ; (20) felting of fibrils between two fibres. It is concluded that (a) it is possible to delignify the fibres of wood in such a way that they can partially felt without the use of a powerful tearing action ; (b) suitable paper properties can only be obtained by the use of more than one piece of plant. The author proposes the use of a beating process using a plant having a number of small discs of very high resistance and of high precision but low power made from a metal. Sixty-five references are given.

PATRA.

REVIEWS.

The Bacterial Cell in its Relation to Problems of Virulence, Immunity and Chemotherapy.—RENE J. DUBOS. XIX, 352, pp., 33 figs. Havard Univ. Press. With Addendum :—

Nuclear Apparatus and Cell Structure of Rod-shaped Bacteria.—C. F. ROBINOW, 355–77, 8 pls.

Bibliography. 379–449 ($8\frac{1}{4} \times 7\frac{1}{2}$).

This volume which deals with practically every aspect of Bacteriology commences with the cytology of the organism, physicochemical, and staining properties. The action of enzymes is discussed.

Immunochemical analysis and the variability of bacteria is treated at length and considerations of bacteriostatic and bacteriocidal agents comprise Chapter VIII.

The first part concludes with a chapter on " Trends and Perceptions " in which the development of knowledge concerning bacteria, nature of specificity, and significance

of bacterial variability. The figures include photographs of gross variations appearing in Perri-dish colonies with some of the respective organisms. Several electron micrographs are included and some of the best shots from the films of Dr. Adrianum Pijper exhibiting the supposed "motile" organs or "Flagella" of bacteria taken by the sunlight darkground technique.

The addendum deals with the nuclear structures of resting bacterial spores as well as those of the adult cell: changes taking place during growth are shown in seventy-five different photomicrographs.

This is an able book which, although not by any means a complete text-book, serves as a rather complete résumé of the subject; it should stimulate research in more than one direction.

F. C. G.

Cytological Technique.—L. F. La COUR ("Improvements in Plant Cytological Technique," 1947, *Bot. Rev.*, **13**, 216–40). A comprehensive review of the most modern and successful techniques is undertaken and a very complete bibliography is included together with schedules for many of the processes. Much of the paper relates to squash and smear methods particularly by the Feulgen technique. Useful comments are added for guidance in the course of the work.

The writer makes a point of Johansen's remarks on the use of 2BD and 2BE fixatives (La Cour) giving atrocious results on the Pacific coast and a claim that "in the hands of capable workers" in this country no such difficulty is experienced, he does not appear in the paper to have examined the reasons for the divergence of opinion in this respect. The author also claims the ethyl alcohol-chloroform method preferable to all others for the paraffin process; the cellophane square used for germinating pollen grains and subsequent treatments are described, it is used both for the acetic lacmoid and Feulgen techniques.

Special treatments for revealing spiral structure are briefly dealt with and the paper ends with a few remarks on Histo-chemical tests and some formulæ in addition to earlier work. The references number seventy-six.

F. C. G.

"Nucleic Acid," No. 1. Symposia of the Society for Experimental Biology.—Twenty-five Authors, nineteen Chapters and Preface by J. F. DANILI and R. BROWN, Hon. Secretaries, Society for Exp. Biol. 1947. vii+290 pp. Cambridge Univ. Press. Price 35s.

A very comprehensive treatise on every aspect of the relationship of nucleic acid to cellular metabolism and the subject is treated from the purely chemical aspect to considerations of the gross morphology of chromosome growth and change.

This number appears to be the initial stage, in the words of one author, to re-write cytology, for in the light of the papers by the various contributors this is shown to be plainly necessary, that the old empirical methods are giving way to more exact chemical analysis and the results of X-ray experiments revealing fresh viewpoints signify deep-seated changes that are making themselves evident.

The book opens with two chapters on the "Structures of the Nucleic Acids" and the "Structure and Synthesis of Nucleotides." It is very clear that the improved methods and discoveries of the past decade have presented an entirely fresh viewpoint to biological investigation the chemist must walk closer with the biologist and *vice versa*. A picture arises from the period of large complicated molecules built up of hundreds and thousands of nucleotides into polynucleotides united through phosphoester linkages of the same type of linkage as those uniting the component nucleotides.

The author contends that no evidence appears to have been obtained that the nucleotides are arranged in any other than a random manner in polynucleotides. Concerning an approach to synthetic methods a start would be made with the smallest units (nucleosides) and from them build up to more complicated structures: the problems are enumerated as follows: (a) synthesis of nucleosides; (b) phosphorylation of sensitive molecules (nucleotide synthesis); and (c) Linkage of molecules through phosphate and polyphosphate residues (polynucleotide synthesis).

The third chapter stresses the importance of finding a solution to the problem by which the nitrogenous bases and their ribosides which constitute the "building blocks" of nucleotides and nucleic acids are synthesized, and to understand on a chemical basis the biological action of the nucleic acids. The value of micro-methods for this type of analysis is dealt with.

The macromolecular behaviour of desoxypentose nucleic acid obtained from calf thymus gland is treated and details of work proceeding which should yield much of interest are included.

X-ray studies of nucleic acids reveal figures of high density, for, on drying *in vacuo* over P_2O_5Na thymonucleate gives a value of 1.63 g./c.c. and yeast nucleic acid and tobacco mosaic virus nucleic acid 1.65 g./c.c. Evidence is submitted as to the regularity of the tetranucleotide constitution of the molecule by means of X-ray fibre photographs.

The distribution of nucleic acids in tissues and the relative amounts of the two types is considered together with the effects of physiological activity. Questions arise in bacteriology both on the structure of the nuclear material and the nucleic acid and the nucleoprotein content of the various forms.

Cytochemistry forms the subject of the eighth section and divides the problems into three groups: (i) distribution of concentrations of various chemical species in both normal and pathological cells; (ii) the velocities of chemical reactions proceeding in the cell, and mechanisms of storage and mobilization and organization of energy on the chemical level; and (iii) determine the mechanism of mobilization and organization of energy on a micro- and macroscopic scale and provide, on the cellular level, an explanation of the relationship between morphology and function.

The relationship between ribonucleic acid and the vacuoles are considered and evidence shows that Golgi vacuoles may contain small quantities of ribonucleoproteins. Nucleic acid and protein synthesis are investigated by ultra-violet methods.

Under nucleic acid in nerve-cell regeneration it is revealed that in the regenerating neuron the morphological, physiological and chemical changes show a reversion towards the level of the embryonic period and return to adult levels as regeneration is completed. Experiments on tumour tissue in mice show that purine compounds, adenylic, and guanylic acids, cause an inhibition of tumour growth. The relative amounts of thymonucleic over ribonucleic acid in homologous normal and neoplastic tissues show an increase in the latter. Ribonucleic acid was increased in about half the instances measured; there is, it is stated, insufficient evidence to accept as final any of the interesting possibilities that appear to arise from these facts.

Embryological material is adequately treated as likewise the action of enzymes on the chromosomes. The function of deoxyribose nucleic acid in the nucleus is critically considered, four elements are enumerated as permanently attached to the chromosome fibre: the centromere, the euchromatin and heterochromatin, and the nucleolar organizer which is the locus responsible for the collection of nucleolar material. The relationships of some of these elements is debated. Nucleic acid starvation and chromosome breakage produce interesting results.

The final chapter treats of the experimental modification of nucleic acid systems in the cell and shows that the nucleoprotein on the chromosome participates in nucleic acid synthesis and controls it. Heterochromatin behaves as an indicator of metabolic conditions within the cell and seems to be responsible for maintaining the equilibrium between demand and supply for nucleic acid.

The above notes, necessarily brief, serve to summarize the embracing scope of the work which could with advantage be copied in other spheres of scientific thought. Each author is a master of the subject and, although opinion varies and even differs fundamentally in certain instances, this should stimulate further work for anyone who reads it.

F. C. G.

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TRANSACTIONS OF THE SOCIETY.

I.—THE STUDY OF LIVING MALIGNANT CELLS BY PHASE-
CONTRAST AND ULTRA-VIOLET MICROSCOPY.

By R. J. LUDFORD.

(Mount Vernon Hospital and The Radium Institute.)

J. SMILES and F. V. WELCH.

(From the National Institute for Medical Research, London, N.W.3.)

TWO PLATES.

Few would dispute the contention that the best way to investigate the mechanism of life is to study living cells. Recent advances in microscopy have gone a long way to make this possible. Living cells, whether spread out in tissue cultures, or flattened out from tissue smears, are too transparent to make possible detailed observations by transmitted light. The differences in refractive index between the various structural components of cells are insufficient to render them distinct in the microscopic image, since they also lack specific absorptions in visual light. These difficulties have been overcome by the introduction of the phase-contrast technique, which accentuates differences in refractive index. Credit for its inception is due primarily to Zernicke (1935a, b), and its development owes much to the work of Loos (1941). In our investigations we have employed the apparatus which was designed by Taylor (1946), to whose paper reference should be made for the theory and practical applications of this new technique.

Phase-contrast microscopy reveals living cells with a clarity only previously obtainable with the more exacting cytological techniques without necessarily increasing resolution. Theoretically this is only possible by employing light of shorter wave lengths. Hence the advantage of ultra-violet micrography which

approximately doubles the resolution of visual microscopy. The first ultra-violet micrographic equipment was designed by von Rohr (1904) and Köhler (1904), and this type of microscope was developed to a high degree of efficiency by Barnard in the National Institute for Medical Research. We have used the Barnard apparatus and employed the technique devised by him and Welch, (Barnard and Welch, 1936a, b). Since their work was concerned mostly with bacteria and viruses, they employed dark-ground illumination with visual light for selecting objects for ultra-violet micrography. This method is far from ideal when dealing with living cells. With high-power objectives cytoplasmic granules, including mitochondria, are revealed with great brilliance, but nuclear structures are obscured (Ludford, 1935). Chromosomes of dividing cells scatter no light, and are discernible with difficulty as ill-defined opacities within a shimmering mass of cytoplasmic granules and filaments. No mitotic spindle is visible. Hence considerable experience is required before the various phases of the mitotic cycle can be distinguished with any degree of certainty in the unfamiliar dark-ground picture. The ideal technical procedure would be to select cells for study by phase-contrast microscopy and photograph by this method before commencing ultra-violet micrography. As this has not been possible at the present stage of our work we have been reduced to the necessity of studying cells by the phase-contrast microscope, and then selecting others as near as possible in the same physiological condition for examination by the ultra-violet technique. Such a comparative study is essential as the ultra-violet irradiation is injurious to living cells. It thus becomes necessary to correlate the appearances seen in the ultra-violet micrographs with the structures demonstrable by light microscopy. Obviously there is no advantage in greater resolution, if living cells cannot be subjected to ultra-violet irradiation for a sufficient length of time to obtain a satisfactory micrograph, without the induction of pathological changes. When working with living tissues great caution will always be required in interpreting microscopic structures revealed by ultra-violet irradiation when they are below the limits of resolution of the ordinary microscope.

Apart from increasing the limits of microscopic resolution, the ultra-violet technique has the conspicuous advantage of affording information about the chemical constitution of the structures which it reveals. Certain organic compounds possessing specific molecular configurations exhibit selective absorption towards ultra-violet radiations. Thus, Wyckoff and Ebeling (1933-4) working with living grass-hopper spermatocytes found that all cell structures, including the chromosomes, were as transparent to wave lengths of 3500 Å and higher frequencies as they were to visual light. With wave lengths between ca. 2500 Å and 2800 Å, nuclear chromatin exhibited strong absorption, while in the 2313 Å region there was considerable absorption by both nucleus and cytoplasm. According to Caspersson (1936) the ultra-violet absorption of nucleic acid is in the region between 2500 Å-2700 Å, with the maximum around 2600 Å, while proteins of the serum albumen type have their maximum at approximately 2800 Å. The absorption of the former is determined by the nitrogenous bases—pyrimidines and purines—while the absorption of proteins is the result of the presence of the amino-acids, tyrosine, tryptophane, and to a lesser extent

phenylalanine. These amino-acids, which determine the selective absorption of proteins, exhibit a significantly weaker absorption than the nitrogenous bases of the nucleic acids. The absorption of nucleic acid (thymus-nucleic acid), is at the maximum 30–60 times greater than that of protein (serum albumen). The absorbing amino acids occur in the known proteins in a concentration of approximately 5 to 7 p.c., but the purine and pyrimidine bases constitute about 30–40 p.c. of the nucleic acids. Hence when nucleic acids and proteins occur in comparable concentrations, the former dominate the absorption. So it is generally justifiable to assume that cell structures which exhibit marked absorption around 2600 Å contain nucleic acids. No distinction, however, can be made between the two types of nucleic acids—desoxyribose and ribose. The former occurs only in the nucleus, and can be demonstrated by the Feulgen staining method. During cell division it is confined entirely to the chromosomes. Ribose nucleic acid is found in the nucleolus, and is a constituent of certain cytoplasmic particles—microsomes and mitochondria (Claude, 1943).

The general significance attributed to nucleic acids in vital processes may be summarized very briefly as follows. Living matter is characterized by its capacity for self-reproduction. "This property implies that certain molecules or molecular systems build up new ones, identical with the original systems." Such self-reproducing systems comprise proteins associated with nucleic acids. The cell nucleus is the centre of protein metabolism, and nucleic acids are essential for all intra-cellular protein syntheses. (Caspersson, 1936, 1941; Caspersson, and Santesson, 1942).

Investigations of living invertebrate cells by the ultra-violet technique were carried out by Lucas and Stark (1931), Wyckoff and Ebeling (1933), Caspersson (1936), and Harvey and Lavin (1944). Amphibian cells were studied by Köhler (1904), and Wyckoff, Ebeling and Ter Louw (1932) worked with living chick cells. There is unanimous agreement about the advantage of the technique for demonstrating nuclear structures and chromosomes. There are, however, considerable discrepancies in the descriptions as to the cytoplasmic structures which are demonstrable. Some of the micrographs of Lucas and Stark (1931) show mitochondria in grasshopper spermatocytes, but none are visible in dividing cells at metaphase, anaphase or early telophase. The wave-length of the irradiation that was employed was not specified. Also working with similar cells, Wyckoff and Ebeling (1933–4) found that "for light of a wave-length between ca. 2500 Å and ca. 2800 Å formed chromatin is the only strongly absorbing cell constituent." Concerning chick fibroblasts and macrophages in tissue cultures, and erythrocytes and lymphocytes from blood, Wyckoff, Ebeling and Ter Louw (1932) reported that all the gross structures revealed by Feulgen staining were demonstrable in living cells as the result of their intense ultra-violet absorption. "No significant differences were discovered between the living and the fixed preparations" and it was concluded that "ultra-violet microscopy can be considered as the equivalent of a vital staining specific for formed chromatin."

The micrographs published by Harvey and Lavin (1944) of the living egg of the Echinoderm, *Arbacia punctulata*, before and after fertilization show considerable absorption in the cytoplasm with a wave length of 2537 Å. After eggs

had been centrifuged it was found that the clear area which represents the matrix or ground substance of the uncentrifuged egg showed the greatest absorption. Intense cytoplasmic absorption has also been demonstrated in sections of embryonic cells (Caspersson and Thorell, 1941), nerve cells (Landström, Caspersson and Wohlfart, 1941), and serous glands (Caspersson, Landström-Hydén and Aquilonius, 1941). In each case ribose nucleic acid was held to be responsible.

Considerable surprise was aroused more recently by a communication from Brumberg and Larionow (1946). They worked with living cells in tissue cultures and focussed their microscope with visual light as we have done, so that no ultra-violet radiation reached the cells prior to the taking of micrographs. They reported that when living mouse mammary carcinoma cells, and mouse and chick fibroblasts were studied, only nucleoli and the cytoplasm of cancer cells revealed moderate absorption in the 2540–2750 Å region. The cytoplasm of cancer cells which had been vitally stained with neutral red lost its capacity for absorption, which was then localized in the dye droplets. A preliminary exposure of cultures to direct ultra-violet irradiation for 2 minutes produced definite cytological changes. Nuclei then acquired the property of absorption, and micrographs presented the same features as those published by Caspersson. On the basis of these observations it was suggested that desoxyribose nucleic acid is present in the nuclei of living cells in a form which does not absorb irradiations in the 2600 Å band, and that absorption only occurs in injured and dead cells. Were this contention confirmed the value of the previous work would be extremely dubious and the conclusions drawn therefrom invalidated.

Tumours Studied and Method of Examination.

For this investigation a variety of transplantable tumours have been employed. These tumours have been transplanted in the Laboratories of the Imperial Cancer Research Fund over a number of years, and have constituted the experimental material of an investigation into stromal cancer (Ludford and Barlow, 1944, 1945). These tumours have included sarcomas, and mammary and lung carcinomas. Most of the present work has been carried out with a rapidly growing sarcoma of a highly dedifferentiated type in R III strain mice. Its cells present the characters of primitive embryonic mesenchyme. When the sarcomatous transformation of the stroma of the mammary tumours occurred in the first instance there resulted a spindle-celled sarcoma. During its transplantation a further change took place resulting in a more rapidly growing tumour. The cells had apparently undergone a further degree of dedifferentiation and had become considerably larger. The whole tumour was of such a soft consistency that for the purpose of our present investigation it was only necessary to remove a small fragment of it on the tip of an iridectomy knife, place it in a drop of Ringer solution on a slide, add a cover-glass and apply gentle pressure until the required degree of flattening was attained. The highly-fluid viscous nature of the cells enabled them to be spread out so as to form a film, like extensively spread cells in a tissue culture. With other tumours of a more solid consistency small fragments of healthy tissue were removed and cut into fine fragments under a dissecting microscope. These were then flattened out in the same manner. The process of preparing specimens for examination was

carried out as expeditiously as possible. Mice were killed, the tumours exposed, fragments removed and mounted for examination within a few minutes. Microscopic examination was carried out at room temperature. With the phase-contrast microscope relatively prolonged observation was possible, but when working with ultra-violet radiations no one area of a preparation was exposed for more than a few seconds.

Cytology of Living Malignant Cells.

The appearances presented by living malignant cells of the R III sarcoma by phase-contrast microscopy are shown in Plate I. Ultra-violet micrographs of cells of the same tumour at comparable stages of growth are reproduced in Plate II.

The distinctive features of the phase-contrast technique are the clarity with which mitochondria and cytoplasmic granules are revealed; also, the opportunity afforded of following the behaviour of the chromosomes throughout the various phases of mitosis into their functionally active condition in the "resting" nucleus. Thus is eliminated the necessity of resorting to fixation and staining methods, which have been so often accused of inducing artifacts.

Fig. 1, Plate I shows one of the largest of the sarcoma cells. It demonstrates the hyperchromatinic condition of the nucleus, the presence of several nucleoli, and the "nucleolus-associated chromatin" of Caspersson (1947). In addition to the numerous filamentous and granular mitochondria surrounding the nucleus, there is a finely granular cytoplasmic mass towards the upper pole of the nucleus with which there appears to be associated a canalicular or vacuolated system, suggestive of the Golgi Apparatus of osmic or silver preparations. This has not been observed in all cells, but may nevertheless be present, being either hidden underneath the nucleus, or flattened out on its surface so as to be indistinguishable.

The formation of the chromosomes in preparation for cell division can readily be observed. A late prophase is represented in fig. 2. Here the nuclear membrane has just broken down, releasing the chromosomes into the cytoplasm. The mitochondria have become rounded, which is a characteristic feature of so many dividing cells. The chromosomes give the impression of having a fluid gelatinous consistency. They are easily displaced at this stage by pressure on the cover-slip and can be flattened out without revealing any evidence of their internal structure. The mitotic spindle is not distinguishable at any stage of division, although it becomes quite obvious after treatment with certain fixatives, without staining.

Cleavage is illustrated in fig. 3. Most of the mitochondria are still granular. Two large bleb-like masses of homogeneous cytoplasm are indicative of the peripheral bubbling which is characteristic of the telophase of mitosis. Chromosomes still remain distinguishable in the two daughter nuclei. As the cells draw apart the mitochondria revert to the filamentous form and in most cases they appear to be carried round towards the surface of cleavage by streaming movements in the cytoplasm. The latter increases in volume and the number of

mitochondria is considerably augmented before the nuclei have attained the structural reorganization of the "resting" condition.

Direct observation of living cells by the ultra-violet microscope is not practicable. Sufficient details are not perceptible with a fluorescent eyepiece. Hence cytological study can only be pursued by means of micrographs. It has been found possible to take pictures of living malignant cells by employing objectives computed for use with wave lengths of 2570 Å and 2750 Å, without any appreciable injury to cell structures.

Fig. 1 (pl. II) is a micrograph of a sarcoma cell obtained with a wave-length of 2570 Å. The same structures are revealed inside the nucleus as by the phase-contrast method (pl. I, fig. 1), but the advantage of the increased resolutional is obvious in the greater precision with which the finer chromatin granules are resolved. The most intense cytoplasmic absorption occurs peripherally, and immediately around the nuclear membrane there is a clearer region. Towards the upper pole of the nucleus on the right side there is a granular cytoplasmic mass similar to that seen in fig. 1 (pl. I) which it was suggested might be associated with the Golgi Apparatus. The mitochondria in closest proximity to the nuclear membrane exhibit less absorption than those furthest away. Microspectrographic analysis of living cells could not be carried out. The necessary series of exposures to radiations of different wave lengths is not tolerated. On the basis of the work previously discussed there is justification for assuming that substances which absorb strongly in the region of 2600 Å contain nucleotides. Fig. 1 (pl. II) might therefore be interpreted as demonstrating that the concentration of nucleic acid (ribose nucleotides) is highest in the cortical part of the cytoplasm, and that the amount present in the mitochondria may vary considerably, possibly in relation to their state of functional activity. The cytoplasm of these cells fails to stain by the Feulgen technique, and presumably contains no desoxyribose nucleotides. Micrographs of cells at prophase reveal the chromosomes as double filaments, exhibiting intense absorption in the 2570 Å and 2750 Å bands. Fig. 2 (pl. II) shows a cell at early metaphase which has been pressed flat, so as to spread out the chromosomes as much as possible without actually disrupting them. This micrograph was taken in the 2570 Å band. The chromosomes disclose no internal structure, but in places there are indications of their double nature. Since they stain intensely by the Feulgen method at this stage, and show marked absorption in the 2600 Å region, it may be deduced that they have a high content of desoxyribose nucleic acid. This would undoubtedly tend to obscure in ultra-violet micrographs a less strongly absorbing protein structure upon which the nucleic acid may be deposited, or within which it may be incorporated.

Micrographs of cells taken after the breakdown of the nuclear membrane show a definite alteration in the distribution of the cytoplasmic absorbing substances (ribose nucleic acid). As is seen also in the phase-contrast picture, fig. 2 (pl. I), the mitochondria contract forming shortened rodlets or granules. In fig. 2 (pl. II) they are seen to have a peripheral layer of nucleic acid. The cytoplasm itself contains little or no absorbing material. It appears to have been deposited on the surface of the mitochondria. That the ribose nucleotides of the cytoplasm have been converted to the desoxyribose type for building up



FIG. 1.



FIG. 2.



FIG. 3

FIG. 1.

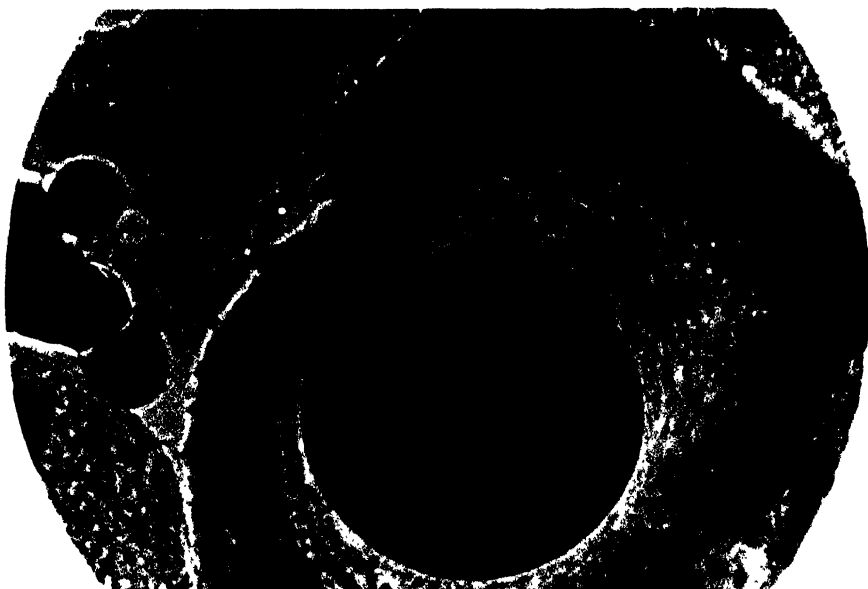


FIG. 2.

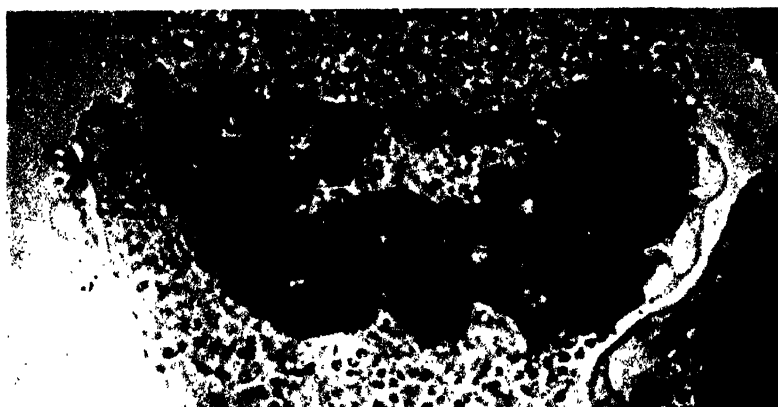
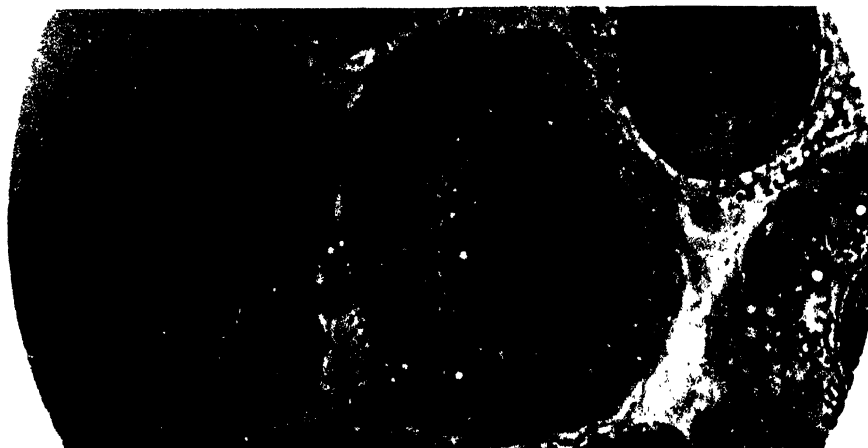


FIG. 3.



the chromosomes seems unlikely. Other micrographs demonstrate considerable absorption by the cytoplasm at prophase, when clearly defined chromosomes are present within the nuclear membrane.

Towards the end of the cleavage process the mitochondria elongate and absorbing substance reappears in the cytoplasm. Two daughter cells separated except for a slender strand of protoplasm are shown in fig. 3 (pl. II). The chromosomes are still easily discernible. No nucleoli are yet formed. Nevertheless the cytoplasm of both cells has re-acquired a considerable amount of absorbing material (ribose nucleotides). It is more concentrated around the periphery of the cells than immediately around the nuclei. A series of micrographs of cells at the end of the cleavage process demonstrate that this is the period of mitochondrial duplication, which is intimately associated with the growth of the cytoplasm.

The chromosomes in fig. 3 (pl. II) present the appearance of double threads. The same structure is seen in cells before the chromosomes have completely separated into two groups at early telophase. This double structure persists into the "resting" nucleus, which is made up of numerous finely attenuated double-threads along the length of which are disposed minute granules not properly resolved with a wave length of $2570 \text{ \AA}$. These minute particles might represent the genes. The detailed study of the living nucleus that is made possible by ultra-violet microscopy opens up new fields for investigation, and this aspect of the subject is being dealt with in another paper.

DISCUSSION.

The primary aim in presenting these results has been to demonstrate the opportunity afforded by these new techniques for the study of living cells. Comparison of the figures of Plates I and II provides means for estimating the validity of ultra-violet micrography as a technique for the elucidation of intracellular processes. The essential identity of all fundamental features in the two sets of pictures should be borne in mind in comparing our results with the ultra-violet technique with those of previous investigators whose work was discussed in the introductory paragraphs. Certain obvious differences are attributable to the great care taken in the present work to avoid exposing cells to either light, or more particularly ultra-violet irradiation, any longer than was absolutely necessary. Mitochondria were found to be extremely sensitive to ultra-violet radiations. They may be completely obliterated by exposures which produce no obvious changes in nuclear structure. The absence of mitochondria from micrographs may therefore generally be assumed to imply that the cells have been seriously damaged by the irradiation.

Since comparison of our ultra-violet micrographs with those of previous workers reveals fundamental differences it is desirable to consider briefly to what extent our results support current theories on the role of nucleic acids in cell growth. With the general conception of Caspersson concerning the relation of nucleic acids to protein synthesis there can be no disagreement. But his theory of the "cytoplasmic protein forming system" cannot be sustained. Briefly Caspersson's scheme is as follows. A certain part of the nuclear

chromatin (nucleolus-associated chromatin) secretes substances of a protein nature. "These substances accumulate and form the main bulk of the large nucleolus. From the nucleolus they diffuse towards the nuclear membrane, on the outside of which an intense production of ribose nucleotides takes place. At the same time the amount of cytoplasmic proteins increases"—(Caspersson, 1947).

Although we have taken ultra-violet micrographs of living cells of three sarcomas and four carcinomas, in no instance has the concentration of cytoplasmic absorbing substance been highest directly around the nuclear membrane, nor has there been any indication of a diffusion of nucleic acid from the nucleus into the cytoplasm. The nucleus is more often surrounded by clear cytoplasm. Then at the telophase of mitosis the cytoplasm begins to exhibit strong absorption before the chromosomes have reverted to the condition characteristic of the "resting" nucleus, and previous to the formation of nucleoli. The appearance presented by our micrographs suggests that cytoplasmic protein synthesis occurs in the more peripheral part of the cell and that mitochondria are involved in the process. The degree of absorption, by mitochondria, of radiations of wave lengths between 2570 Å and 2750 Å varies. In the same cell some mitochondria may absorb strongly, others very weakly. Absorption also varies from cell to cell of the same tumour. Presumably mitochondria segregate, or condense on their surfaces, varying amounts of ribose nucleic acid according to their state of functional activity. It is possible that the mitochondrial-cytoplasmic interface may be the site of cytoplasmic protein synthesis. Such a conception would not conflict with what is known of the behaviour of mitochondria under varying physiological and pathological conditions.

In conclusion it should be emphasized that the sarcoma represented in our illustrations is the most rapidly growing mouse tumour at present available. When ultra-violet micrographs of this sarcoma are compared with those of the other tumours, the most striking difference is in the amount of cytoplasmic absorbing substance (ribose nucleotides). In general, the faster growing the tumour the more intense is the cytoplasmic absorption of radiations of wave lengths around 2600 Å. Fibroblasts of subcutaneous connective tissue and mesothelial cells have shown the least absorption: the greatest has been exhibited by our fastest growing sarcoma, that illustrated in Plates I and II.

Finally it might be pointed out that the microscopical techniques which we have employed here for a problem of cancer research are equally applicable to a much wider field of investigation in both biological and medical research.

SUMMARY.

(1) By phase-contrast microscopy, mitochondria and cytoplasmic granules are demonstrable in living cells with the greatest clarity, and at the same time the behaviour of the chromosomes can be followed throughout the mitotic cycle (Plate I).

(2) Ultra-violet micrographs of living malignant cells obtained with wave lengths of 2570 Å and 2750 Å, show by comparison, no appreciable injury to intracellular structures. (Compare Plates I and II.)

(3) From the distribution of cytoplasmic material exhibiting strong absorption around 2600 Å (ribose nucleotides) in cells at various stages of growth, it is concluded that cytoplasmic protein synthesis occurs in the more peripheral parts of cells, and not in the immediate vicinity of the nucleus. It is suggested that mitochondrial-cytoplasmic interfaces might be the site of protein synthesis.

(4) There is a very considerable difference between the amount of cytoplasmic material exhibiting absorption around 2600 Å (ribose nucleotides) in rapidly growing sarcoma cells, and in fibroblasts and mesothelial cells. Generally, the more rapidly growing the cells the more intense is the cytoplasmic absorption.

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DESCRIPTION OF PLATES.

PLATE I.

Living sarcoma cells (R III Mouse sarcoma) photographed by phase-contrast microscopy. ($\times 2100$.)

Fig. 1.—“Resting” cell.

Fig. 2.—Early metaphase of mitosis.

Fig. 3.—Daughter cells in process of separating.

PLATE II.

Living cells of the same tumour as those depicted in Plate I, as demonstrated by ultra-violet microscopy. ($\times 2650$.)

Fig. 1.—“Resting” cell: 2570 Å.

Fig. 2.—Early metaphase chromosomes which have been compressed: 2570 Å.

Fig. 3.—Daughter cells almost completely separated: 2750 Å.

II.—TISSUE DISINTEGRATOR.

By H. MICKLE.

ONE PLATE.

It has for a long time been a problem to grind tissue without bacterial contamination in a minimum of time. The usual methods of grinding are not ideal for several reasons :

- (1) Grinding is slow and handling times in loading and unloading are long.
- (2) There is an appreciable loss of material.
- (3) Material has to be re-sterilized after grinding.

A problem which is somewhat similar, i.e. that of producing colloidal mixtures by violent shaking, has been dealt with repeatedly in the literature, but in tissue grinding disintegration of the tissue has to be achieved in addition. Most authors who dealt with this subject thought until recently that it was necessary to use supersonics for this purpose, but this was disproved by M. von Ardenne in a paper which was published in *Zeitschrift für Angewandte Chemie*, March, 1941, page 144.

The author proposed to use a tuned reed on to which the vessel to be shaken is attached, the reed being made to vibrate magnetically by coils which are energized from the 50 cycle alternating current supply. M. von Ardenne's results, which were illustrated by photographs, are very striking, so that his method seemed to be very suitable for the purpose of tissue grinding. However, as grinding put an appreciably higher strain on the apparatus than just mixing, it was not clear that this shaker would fulfil the necessary requirements. In order to assure this from the start the stability of the system was improved by the use of two symmetrically arranged reeds similar to a tuning fork. This has also the advantage of being able to deal with two vessels simultaneously.

It has been further observed that it is more advantageous to attach the vessels horizontally, i.e. with their axes in the same plane as the plane in which the vibration of the reeds takes place, because if they are attached with their axes perpendicular to that plane, which seems to be the more natural way, one observes that vortices formed by the superimposed circular movement of the reeds cause the fluid to cling to the wall of the vessels, thus preventing the mixing and disintegration of the material.

Another way to prevent the vortices forming, even when the vessels are attached vertically, was found in providing the vessels with inwardly protruding baffles which break up the vortices.

The grinding and colloidization action takes place through the violent impulse which the liquid gets when the reeds reverse their direction, and it is

enhanced by the difference of the specific gravity of the liquid and of the particles.

Both effects will increase with the amplitude of the reeds. The time taken to get the same grinding effect thus decreases when the amplitude increases. There is, however, a limit to the amplitude because of the serious strain on the reeds, which will eventually break if one goes too far.

It was found that with mains operation, magnetically polarized steel reeds tuned to a natural frequency of 50 cycles and with an amplitude of $\frac{1}{2}$ – $\frac{3}{4}$ inch produced an extremely good grinding effect, as will be seen by the examples given later. The reeds are made from a Special Quality Spring Temper Steel and are 9 inches on their free length and have a cross-section of $1\frac{1}{4} \times \frac{3}{16}$ inch.

The attachment of the reeds had to be designed with special care, it being found that the orthodox clamping arrangement was not favourable if one wishes the reeds to have a reasonable life. Reinforcement on both sides of the reeds with the same material as that of the reeds, and of a length at least equal to half their width, gave best results. The clamping arrangement is designed in such a way that the exchange and tuning of a new reed in case of breakage takes only a minute or two.

The apparatus in its present form consists of a cast-iron clamping-block holding the two tuned reeds, on the ends of which are mounted the light alloy containers into which are inserted the Pyrex glass vessels. Each reed passes through a direct current field-coil, the two coils being wound in opposite directions; thus one reed is polarized so as to give a permanent North pole at its extremity, and the other a South pole. Spanning the two reeds at a predetermined point is an alternating current electro-magnet with a horseshoe-shaped core. From this it follows, that for one half-cycle when the reeds are of the same polarity as the ends of the alternating current magnet core, they are repelled inwardly towards each other, and for the second half of the cycle when the electro-magnet is of opposite polarity to that of the reeds, they are attracted towards the core and away from one another. The amplitude is controlled by means of a rheostat which varies the intensity of the direct current in the polarizing coils. The whole arrangement is mounted on a cast-aluminium base-plate of sturdy construction and enclosed in a duralumin cover. The apparatus illustrated is fitted with containers having an observation window, but where infected material is being ground totally enclosed containers are advisable to prevent spraying of the liquid should a vessel break.

The following two reports illustrate the use of the apparatus:

(1) The machine has been used for the disintegration of various micro-organisms for the purpose of obtaining active enzyme preparations therefrom. Active preparations have been obtained from baker's yeast and Dutch Top Yeast by shaking in the presence of "glow-beads" for 8–10 minutes. *Bacterium coli* suspensions undergo about 80 p.c. disintegration in 25 minutes, but thick suspensions of *Streptococcus faecalis* or *Staphylococcus aureus* required 45–50 minutes. A notable success was obtained with the yeast *Torulopsis pullcherina*, which could not be disintegrated in any of the usual ways, but was broken after 15 minutes shaking. In all cases suspensions of the organisms of dry weight

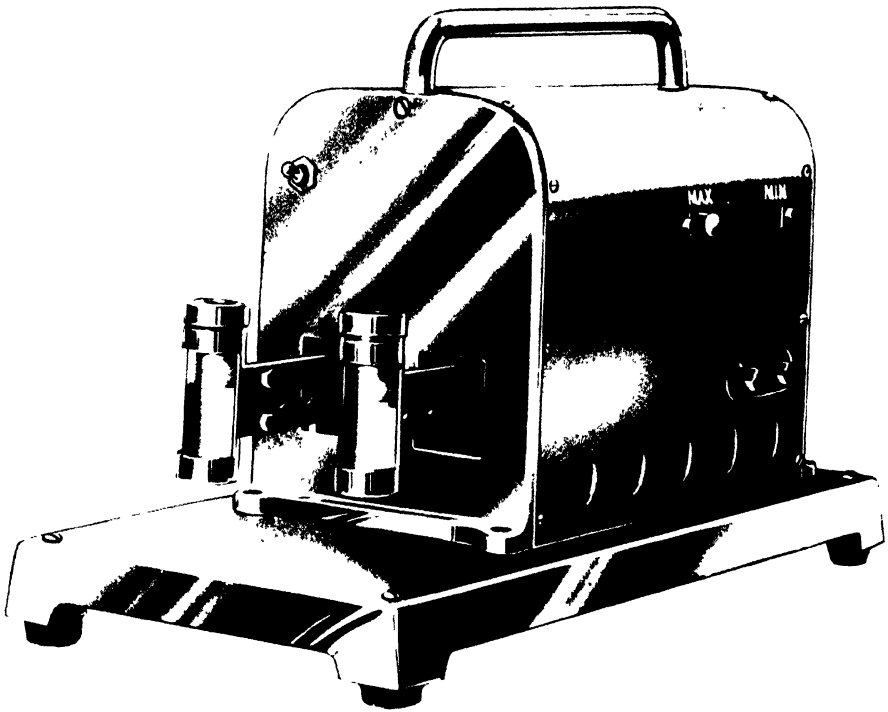
30–50 mg. per ml. were mixed with approximately 30 p.c. their volume of “glow-beads.”

(2) The table below shows the decrease in viable counts of four strains of Group D streptococci when shaken for various lengths of time with carborundum powder (grade 100). After 90 minutes the suspensions were virtually sterile. This method of treatment has proved most effective for the production of suitable preparations of streptococci for the making of potent grouping sera. Group sera for certain streptococci (notably *Strep. bovis*) have proved difficult or impossible to produce by other recognized methods.

TABLE 1.—Viable counts of Group D Streptococci shaken with Carborundum (Grade 100).

Shaking time.				Strain 1.	Strain 2.	Strain 3.	Strain 4.
Initial count	..	..	..	60×10^9	24×10^5	11×10^5	36×10^5
5 mins.	..	..	..	26×10^9	10×10^5	7×10^4	43×10^4
30 „	..	..	..	27×10^5	15×10^3	8×10^3	22×10^3
60 „	..	..	..	4×10^3	7×10^2	16×10	6×10^2
60 „	..	..	..	14	0	3	5

The writer wishes to acknowledge Dr. E. F. Gale, School of Biochemistry, Cambridge University, for his results with yeast; Dr. P. M. F. Shattock, National Institute for Research in Dairying, for her report on Group D Streptococci; and Dr. Kann, formerly of British Coal Utilization Research Association, on whose preliminary design the apparatus is based.



III.—PHASE-CONTRAST MICROSCOPY IN THE STUDY OF FERTILIZATION AND EARLY DEVELOPMENT OF THE RAT EGG.

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(National Institute for Medical Research, Hampstead, London, N.W.3.)

SEVEN PLATES.

INTRODUCTION.

THE existing knowledge of microscopic structure in mammalian eggs is derived almost exclusively from the study of fixed and stained material. Histological methods have much to recommend them in that the final preparation presents a pure colour picture for examination. The eye is most sensitive to colour differences, and fine detail is best appreciated when differentiated in this manner. Furthermore, by the selection of appropriate reagents and stains, useful information is obtained on the chemical nature of the fine structures under observation. Such information cannot be gained by any other method which depends upon visual examination. However, histological methods possess a number of objectionable features, and are limited in their application to dead tissues. The reagents employed cause indeterminate alterations to cells, and doubts have often been expressed as to the true nature of much of the detail described. The satisfactory mounting of mammalian eggs is difficult and time-consuming, and the orientation of microscopic structures within the egg, relative to the plane of section, is usually a matter of chance.

On the other hand, the examination of living eggs can yield little information of value when made with the conventional microscope. In common with other living tissues, eggs are relatively transparent objects and the observation of their morphology by direct illumination depends largely upon the relative refractive indices of the translucent elements of structure present in the ground substance of the cell. When the relative refractive index is equal to unity no reflection or refraction takes place at the surface of the element, which, therefore, cannot be depicted in the microscopic image. In practice this applies also to structures for which the difference in refractive index is small. Beyond this point elements of structure can be depicted by using narrow axial illuminating cones, but the resolution is necessarily small and much detail may become obscured by the presence of undesirable diffraction fringes. As the differences in refractive index increase, wider cones of illumination may be used, giving greater resolution, and the diffraction fringes become less evident. The structures are, however,

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never adequately represented in the image, since they are, when easily resolved, depicted by outlines corresponding to their shape.

Dark-ground illumination, with the high degree of resolution which it allows, has been profitably employed for the observation of many transparent living cells, but this method also gives outline pictures. It has, however, the great merit that structures of low relative refractive index can be made visible provided that the cells are spread out into a thin layer or that the elements are widely dispersed in a thick specimen. These conditions do not apply to mammalian eggs. The structures are closely packed and as the cells are of considerable depth even when manipulated by the method to be described the bright out-of-focus images of structures outside the focussed layer reduce contrast to such an extent that many elements cannot be seen.

Most of these difficulties are resolved by the use of the phase-contrast microscope, and the development of phase-contrast and its application to the microscope by Zernicke (1935), and its subsequent improvement by Köhler and Loos (1941), has placed a tool of great value in the hands of the biologist. The principles and properties of the modern phase-contrast microscope are described in a recent paper by Taylor (1946). It is sufficient to point out here that the important property of phase-contrast lies in the conversion of phase differences into amplitude differences, and that the eye is sensitive only to the latter. Thus, structures which differ in refractive index from the surrounding medium by too small a degree to permit their presence being adequately indicated in the microscopic picture by other methods, are rendered visible by phase-contrast. At the same time, full apertures can be employed and the maximum resolving power of the objectives under the special conditions of illumination brought into use. As a result definition is greatly improved and this, coupled with the small depth of focus, provides a picture which in detail and contrast is rivalled only by the best histological preparations. Furthermore, material can be prepared and examined much more rapidly than by histological procedures. However, in common with all other methods for the study of living material, phase-contrast does not differentiate between structures differing only in chemical nature, and, therefore, to be fully effective it should be used in conjunction with histological methods.

Phase-contrast microscopy finds its most valuable application in the study of small living cells. The mammalian egg is not an ideal object because of its large size, but this difficulty can be overcome, and the account which follows is designed to illustrate the potentialities of the phase-contrast microscope for the examination of this type of material.

APPARATUS.

The phase-contrast microscope used was the standard model made by Messrs. Cooke, Troughton and Simms of York, England. It is fitted with 1.8, 4, 8, and 16 mm. achromatic phase-contrast objectives, and corresponding annular condenser diaphragms mounted beneath the condenser. The intensity of illumination was modified by means of light filters for visual observation and photography.

MATERIALS AND TECHNIQUE.

The rats used were from the hooded and albino strains bred at the Medical Research Council's farm at Mill Hill. The stage of œstrus and the occurrence of mating were determined by means of vaginal smears and the presence of the copulation plug. Frequent examination of the rats during the course of the day enabled reasonably accurate estimates to be made of the time of mating. The eggs were suspended in normal saline, and observed and photographed as soon as possible after recovery.

For the examination of finer structures within eggs it has been found most expedient to press them beneath a cover-slip. The egg is transferred to a slide with a small drop of saline. A thin layer of vaseline is applied to the edges of a cover-slip, the corners being left free so as to allow escape of air. The cover-slip is then placed over the egg and light pressure applied, while the effect on the egg is observed under the microscope. A moderate degree of flattening is usually sufficient and the resulting improvement in visibility can be appreciated by comparing the unpressed 2-cell egg in Plate VI, No. 1, with a similar egg treated as described above (Plate VII, No. 2). It is inevitable that some alteration in the internal arrangement of the egg will result from this procedure, but it has not been found to be of a serious nature. Such alteration as occurs can be watched during the application of pressure, and its significance appreciated. Eggs appear to tolerate the flattening well, for periods up to about 1 hour. After such periods, however, marked changes usually take place, and, within the space of 1 or 2 minutes, vacuolation and increased opacity become apparent in the cytoplasm. These phenomena are also seen when the egg is ruptured by excessive pressure, and are regarded as an indication of cell death.

Pressed eggs can be rolled between cover-slip and slide, and thus be manoeuvred into the most favourable position for examination. This method should be applied with caution as it markedly disturbs the semi-fluid interior.

Eggs surrounded by follicle cells offer a further difficulty, since overlying cells interfere with the definition of structures beneath. It is possible to obtain a relatively clear view in spite of overlying cells if the latter are not too numerous. The ovarian egg in Plate I, No. 1, for example, is seen through the layer of cells shown in Plate I, No. 2. Frequently the overlying cells may be brushed off the zona by side-to-side movements of the cover-slip during the application of pressure. Alternatively, the follicle cells may be removed by treatment with hyaluronidase, if the possibility is born in mind that the enzyme may induce changes in the internal structure of the egg.

OBSERVATIONS.

The Ovarian Egg.—Eggs obtained from the larger follicles in early œstrus have the appearance shown in Plate I, No. 1. The resting nucleus is very clearly defined. It is spherical and the boundary is quite sharp. The eccentrically placed nucleolus is a highly refractile body as may be appreciated from the amount of light refracted on to the overlying cells (Plate I, No. 2). Beside the nucleolus, and apparently attached to it, lies a small dark body. In some eggs

this body is replaced by a large vesicular structure which is very clearly attached to the nucleolus. The white dot within the nucleolus varies in size in different eggs, occasionally having a diameter about one-third that of the nucleolus. The substance occupying the remaining space within the nucleus is structureless and exhibits no indication of the reticulum usually described in fixed and stained material. It is fluid in consistency, for Brownian movement of the small particles present can be observed.

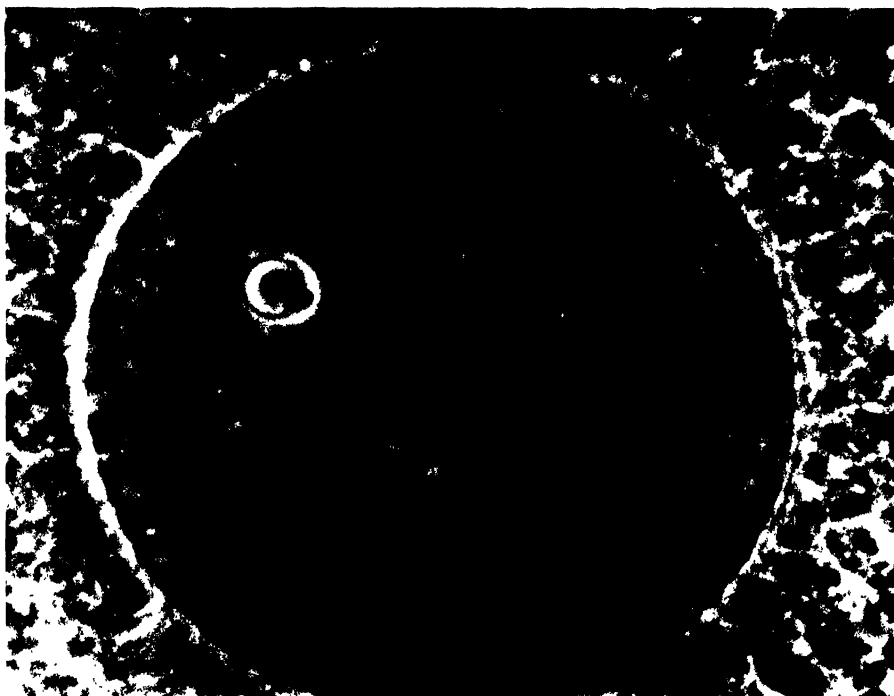
The vitellus provides a complex picture. Most conspicuous are the very numerous small dark granules distributed irregularly in the cytoplasm. The majority of these granules, seen also in the figures as white dots when they are just out of focus, are probably mitochondria. In many places throughout the cell they are gathered into aggregates of varying dimensions, but also form chains. Occasionally granules of larger size are seen, as also are a number of clear bright globules. The nature of these objects is conjectural at the present stage.

Ovarian eggs obtained from 4–10 hours after copulation presented objects resembling the early and late telophases of the first maturation division, which are illustrated in Plate II. Although nearly transparent, the “spindle” behaves as a solid object and is apparently held in relation to a particular region of the surface of the vitellus. When the egg is rolled between cover-slip and slide, the “spindle” retains its orientation to this surface region and moves with it, while the fluid constituents of the vitellus, together with the suspended granular material, can be seen to flow past in a different direction.

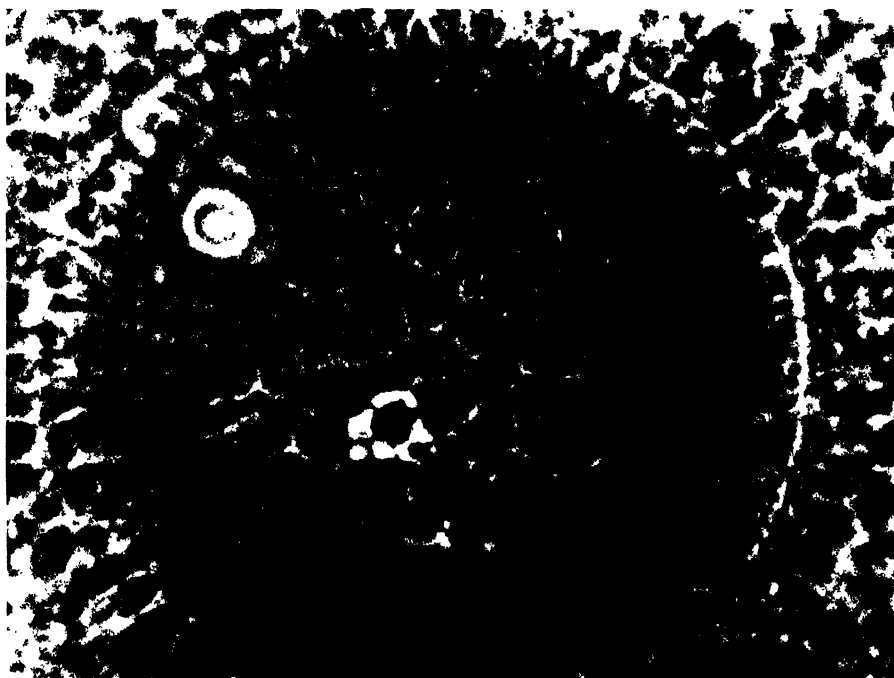
The zona pellucida appears as a homogeneous refractile material. Fine processes from the coronal cells can frequently be seen penetrating the entire thickness of the zona.

The Early Fertilized Egg.—Some groups of female rats were kept with males, examined each morning, and killed when the copulation plug was found. Wide variations were apparent in the state of the follicle cell mass surrounding the eggs from these rats. In a number of instances the cumulus seemed to be quite intact, and could not be distinguished from the cumuli accompanying the freshly ovulated eggs of unmated rats. Such a cumulus is illustrated in Plate III, No. 1. Of the eight eggs discernible here, seven were found to contain sperm within the vitellus. One of the latter is shown in Plate III, No. 2. These eggs were not treated with hyaluronidase nor much compressed between cover-slip and slide. Definition was therefore relatively poor. Nevertheless, the sperm tail and the two pronuclei could be recognized. The entire sperm tail accompanies the head into the vitellus, for the filamentous distal portion can usually be distinguished as it lies amongst the vitelline granules. Another and similar cumulus was treated for a brief period with hyaluronidase solution and the eggs pressed firmly beneath the cover-slip. They presented the same features as those just described, but with improved definition (Plate IV). The significance of these observations with respect to the part played by hyaluronidase in fertilization has been discussed elsewhere (Austin, 1948).

Examination of eggs at a somewhat later stage, in which the follicle cells are dispersed to varying degrees, reveals the rapid growth of the pronuclei to the dimensions shown in Plate V, No. 1. A higher magnification of the larger



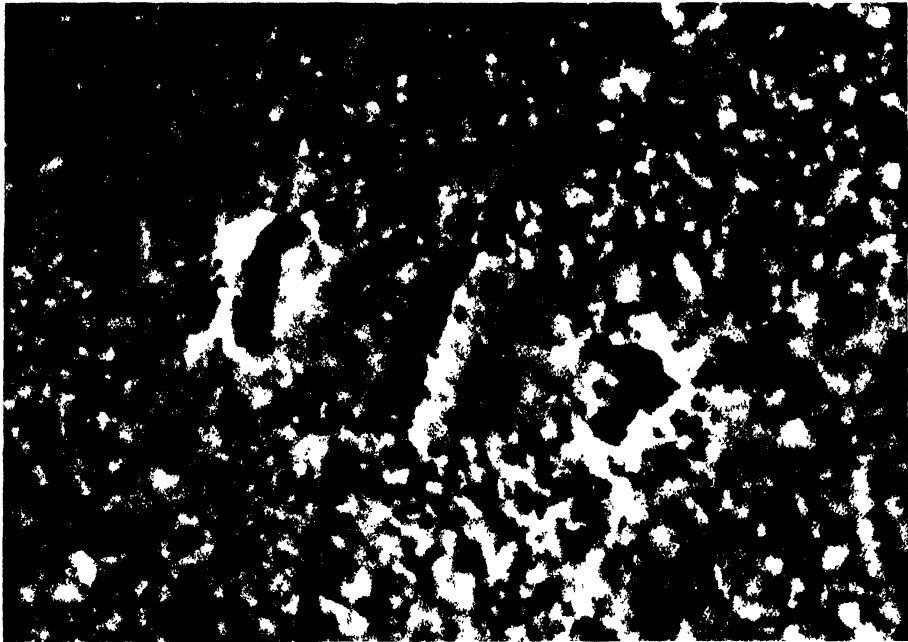
670.



No. 2

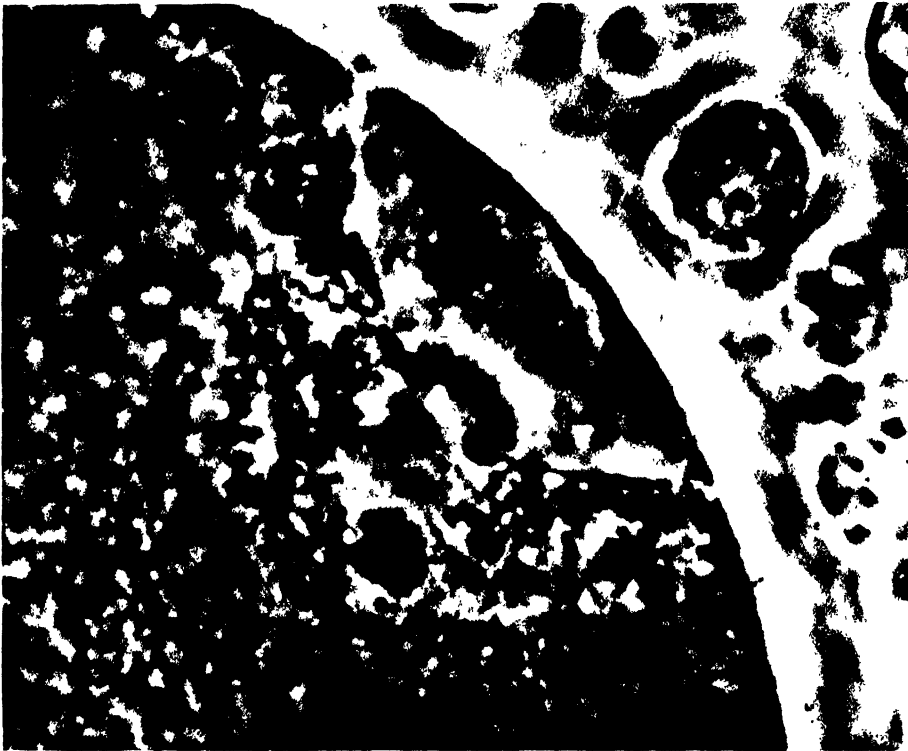
× 670.

[To face p. 16.]



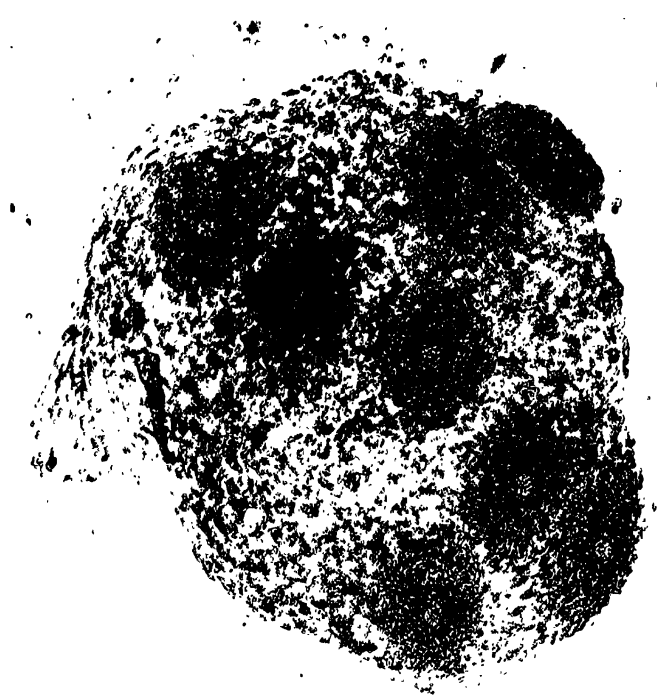
No. 1.

× 2000.



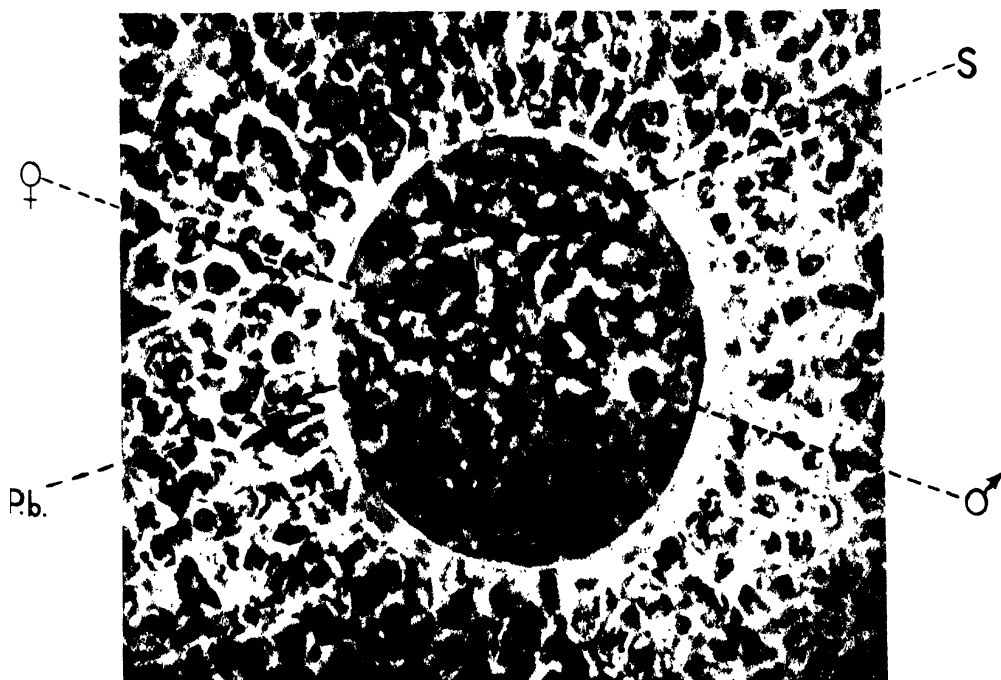
No. 2.

× 2000.



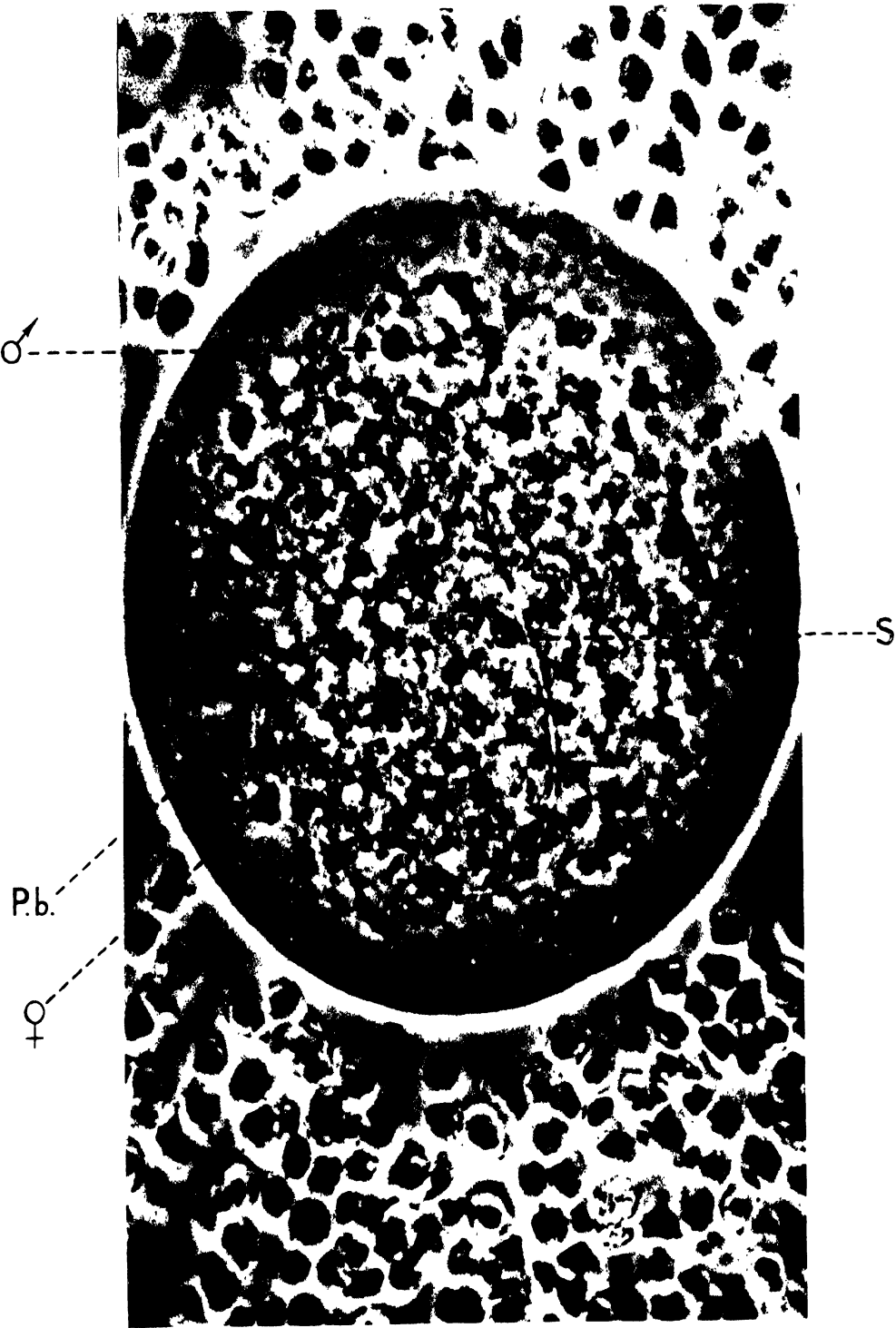
No. 1.

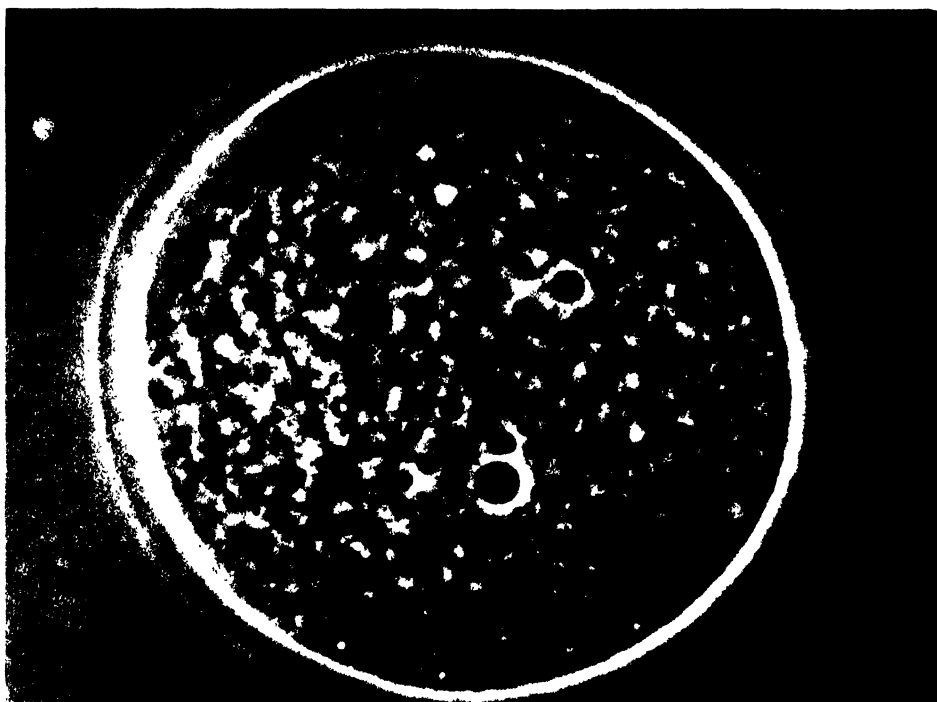
40.



No. 2.

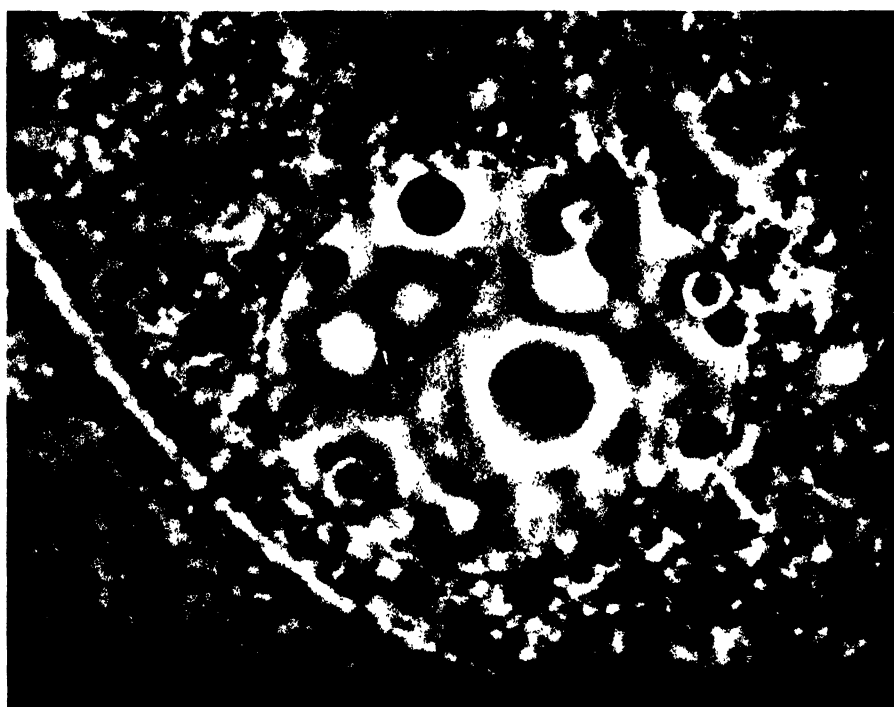
X 450.





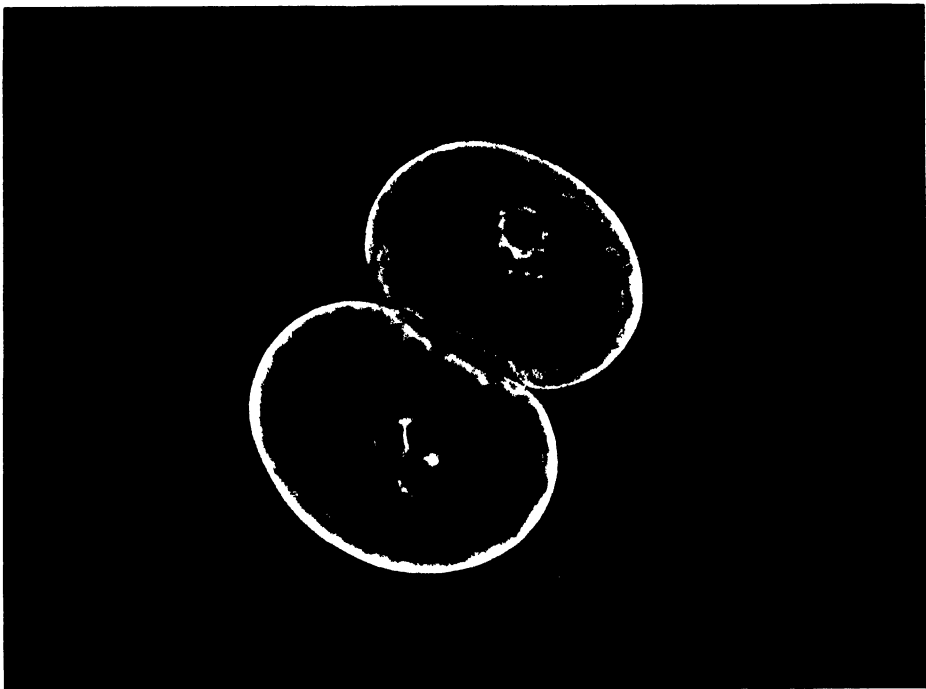
No. 1.

× 750.



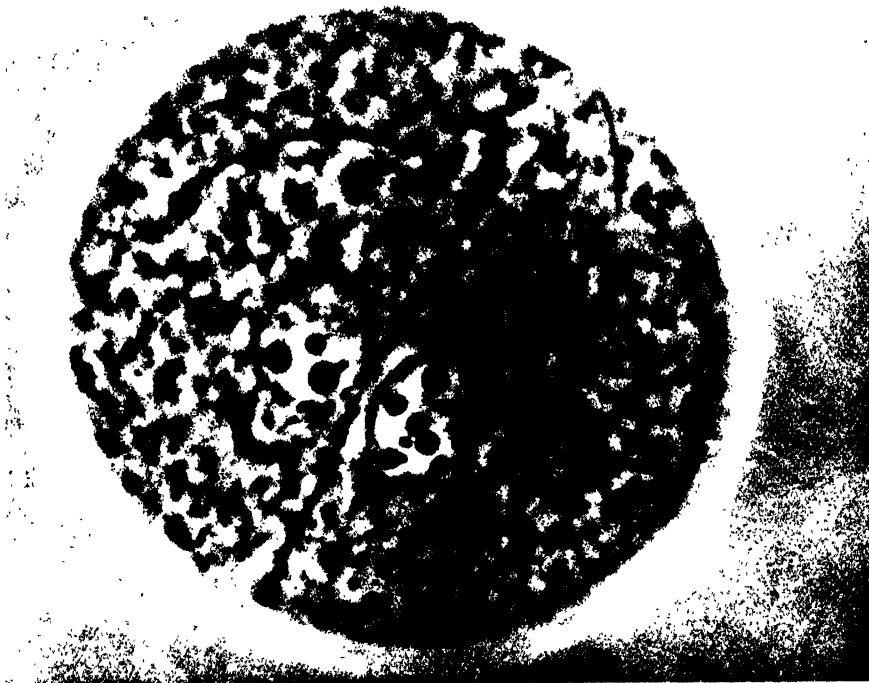
No. 2

× 2500.



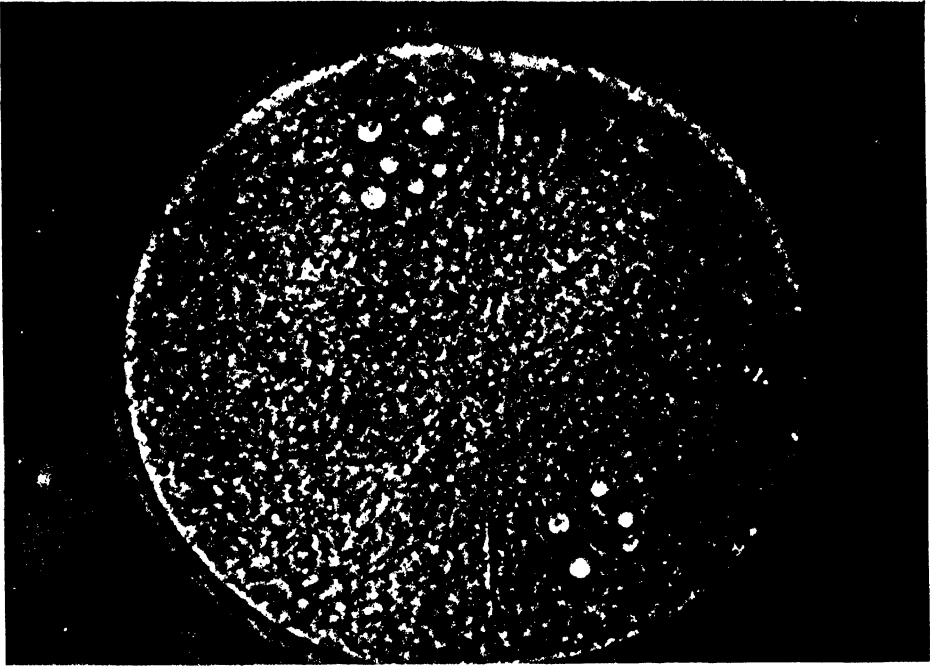
No. 1.

× 650.



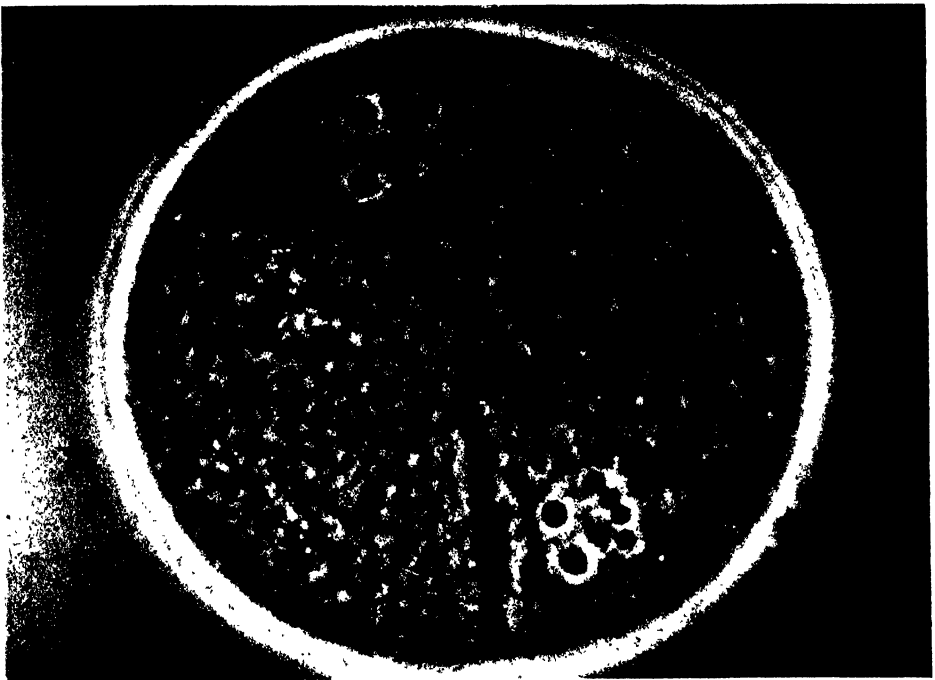
No. 2.

× 750.



No. 1.

750.



No. 2.

× 750

pronucleus (Plate V, No. 2) shows the sharply defined boundary which appears to be a layer of fine granules deposited on the nuclear membrane. The out-of-focus picture of the sperm tail can be seen below and to the left of the pronucleus. The dark spherical nucleoli are a conspicuous feature of both pronuclei. When sharply focussed these globules appear black, but as the focal plane passes above or below them they are replaced by areas of grey, paling off to white. The number, disposition, and relative sizes of the nucleoli vary fairly widely in different eggs, as many as eleven or twelve of moderate size being counted in some pronuclei. The greater part of the space in the nucleus is structureless and is evidently filled with fluid, as in the ovarian egg. Globules similar to those in the pronuclei, though somewhat misshapen, have been observed in the polar bodies. If the egg is broken by heavier pressure on the cover-slip the contents stream out into the surrounding saline and frequently the nucleus passes out intact. Further pressure ruptures the nucleus, the fluid mixes with the saline, but the nucleoli do not, though they occasionally break up into smaller spherical fragments.

The cytoplasm is similar in appearance to that observed in the ovarian egg, with the exception that the aggregations of granules are somewhat larger and more distinct. Frequently these aggregations take the form of hollow spheres.

The Segmenting Egg.—Segmenting eggs (Plate VII, No. 2) do not differ greatly from those in the late unicellular stage, and their nuclei resemble the pronuclei. The sperm tail can still be observed in two- and four-celled eggs. Only one case of polyspermy has thus far been observed (Plate VI, No. 2). In this egg two sperm are present, but no sperm heads are discernible. Just above one of the normal nuclei is a small accessory nucleus which is considered to be a relic of the unused male pronucleus. So that all these structures would be in focus at the same time, the photograph was taken with a low-power objective. Subsequent photographic enlargement has led to a loss of definition.

DISCUSSION.

Some of the properties of phase-contrast can be appreciated by comparing the two-cell egg when photographed by direct illumination (Plate VII, No. 1) with the picture obtained by phase-contrast (Plate VII, No. 2). The two are not strictly in the same focal plane, but even so comparison is quite informative. The former is essentially an outline picture, and this is particularly evident in the boundaries of the nucleoli, vitellus, and zona. The nuclear boundary, however, is scarcely distinguishable, and no pattern can be discerned amongst the vitelline granules. To some extent these shortcomings are due to the relatively large focal depth accompanying a reduced aperture, but the employment of full aperture would result in disappearance of contrast. The use of full aperture with phase-contrast is accompanied by a small depth of focus so that the interference caused by structures above or below the object is not so serious. Thus, the picture in Plate I, No. 1, is seen through the overlying layer of cells shown in Plate I, No. 2, yet interference is not great, and nucleolar details are easily discerned.

IV.—NON-SPECIFIC RESISTANCE AGAINST VIRUS INFECTIONS.

By G. M. FINDLAY.

OUR knowledge of resistance to virus infections is still rudimentary. It is still impossible to state why, for instance, the distemper virus should affect only dogs, foxes, and the weasel family, or why yellow fever should produce necrosis in the livers only of men, monkeys, and hedgehogs, although a very similar mosquito-transmitted virus, that of Rift Valley fever, causes necrosis of the liver in men, monkeys, sheep, goats, cattle, and mice. Even comparatively closely related genera may react in an entirely different fashion to the same virus: the mouse is highly susceptible to many virus infections, the rat to hardly any. Still more puzzling is the fact that some individuals of a highly susceptible species may acquire immunity to a virus without exhibiting clinical symptoms of ill-health. In yellow-fever epidemics it is the rule to find that a considerable number of persons have acquired immune bodies as a result of an inapparent infection. Almost all laboratory workers who have carried out experiments with Rift Valley fever have become infected. The majority have had short febrile illnesses but some have become immune without symptoms of ill-health. Even when immune bodies are present in the bloodstream pathological lesions and clinical symptoms may still develop. A high percentage of the adult population has immune bodies to herpes in the blood, but that does not prevent their suffering from herpetic eruptions when local resistance is reduced by non-specific means.

Folk medicine has always recognized that there are two forms of resistance to virus infections, a specific resistance developed as the result of a previous adequate exposure to a particular parasite and a non-specific resistance which may vary in accordance with factors as yet but little understood.

Specific immunity of long duration has been recognized for many centuries as resulting from one attack of smallpox, chicken pox, or mumps, and the practice of exposing all the children of a family to infection when one of them has succumbed, in order "to get it over," is by no means extinct even in comparatively enlightened communities. Less civilized races have also recognized and made use of the fact that one attack of certain virus infections confers a lasting immunity. The Yoruba, Ewe, and the peoples of Dahomey who inhabit the coastal region of the Gulf of Guinea believe that smallpox is sent as a punishment by the Earth god (Shapona, Shakponon, Sopoton). Those who recover from the infection become devotees of the god and they alone are permitted to nurse and bury the victims of the god's anger: in return for their services they acquire most of the victims' movable possessions. Immunization against smallpox has been practiced by the Chinese for countless generations by insufflating the dried virus into the nose. In the Sudan among the Shilluks and Nuba, Bruce (1790)

described "buying the smallpox" which was then inoculated into the arm and similar procedures are recorded of Somali, Bari and Baganda tribes. The same custom is observed by Sudanic negroes in West Africa: smallpox crusts have a market value and are bought and sold in the Northern Territories of the Gold Coast (Rattray, 1932). There is also some evidence that "buying the smallpox" was practised in Pembrokeshire certainly as far back as the sixteenth century (Williams, 1729a, b, Wright, 1723). The practice of variolation was of course officially introduced from Constantinople into England by Lady Mary Wortley-Montague in 1718. Jenner in replacing variolation by vaccination was merely exploiting the belief long current in the Gloucestershire countryside that an attack of cow pox protects against smallpox. The Fulani, that remarkable non-negroid, cattle-owning race which wanders over the savannah lands of West Africa, evolved a method of immunizing against rinderpest which is only now giving way to the use of goat-adapted virus (de St. Croix, 1945).

In addition to the protection afforded by specific immunization it has also been recognized that non-specific factors are involved in maintaining resistance, as exemplified in the expressions "run down" or "below par."

Physicians in the seventeenth and eighteenth centuries were quite convinced that a mind free from fear was a great protective against infection. De Diemerbroeck (1672) records many instances of persons contracting smallpox "by fear and imagination without any occasion of infection," for "sudden conturbation of the mind and spirits causes a disposition to arise in the body which at length produces the smallpox."

Hume (1780) believed that against yellow fever the best protection was "in the mind being perfectly at ease" for he did not "think anyone in more danger than the timid and pusillanimous, scarcely even the intemperate stranger." Many physicians of to-day who have passed through epidemics would be inclined to agree.

While immunology has now developed into an elaborate science which has successfully evolved methods of immunization against smallpox, rabies, yellow fever, measles, dog distemper, rinderpest, horse sickness, equine encephalomyelitis, louping ill, hog cholera, fowl pox, Newcastle disease, and fowl pest, studies of non-specific factors in resistance have received small attention, largely because they have not yet been correlated to form a consistent whole; nevertheless, the question of non-specific resistance is one which is of considerable importance as a protection against virus infections.

The following factors appear to play a part in non-specific protection: genetic constitution, age, nutrition, hormone production, body temperature, interference by other viruses, specific cell receptors, and the presence of pseudo-virucidal bodies in the serum. A brief discussion of these factors may not be without interest as contributing towards a constructive outlook on the causes of non-specific resistance.

GENETIC CONSTITUTION.

It was at one time thought that the negro was less susceptible to yellow fever than were those with white or yellow skins. Further investigation, however, has shown that the reason for the apparent insusceptibility of the negro is that he

frequently acquires a lasting immunity in childhood.* Epidemics of yellow fever in the Gold Coast in 1937, in the Sudan in 1940, and in Nigeria in 1946 prove that negroes are highly susceptible to yellow fever and succumb to it quite readily. The negro undoubtedly does possess a racial resistance to the parasites of benign tertian malaria. This was demonstrated in the last war in India and Burma when West African soldiers were exposed to infection by strains of *Plasmodium vivax* to which they could never previously have been exposed.

In Africa some strains of humped or Zebu cattle are more resistant than unhumped varieties to the virus of rinderpest. The small forest and lagoon cattle of West Africa derived from *Bos brachyceros* are much less resistant than the zebu or humped type which is probably of Asiatic origin. The virus of rinderpest it may be noted was introduced comparatively recently into Africa from Europe and Asia (Stewart, 1938).

There is some evidence that poliomyelitis attacks most readily those of a certain genetic constitution. Draper *et al.* (1944) as a result of measurements and observations on children attacked with poliomyelitis conclude that the susceptibility to the virus is part of a particular constitutional defect which is probably hereditary. In the New York epidemic of 1916 Lavinder *et al.* (1918) found that the attack rate per 10,000 population of those exposed in the family was 109.1, while among the general population it was 18.4. Probably many of those attacked were related as well as exposed, for 393 of the 407 patients were children under 11 years of age. Infection and exposure were thus both in operation. Aycock (1942) believes that siblings and children of the same parents are more liable to attack irrespective of exposure. Because of the low attack rates of poliomyelitis the development of multiple cases in the same family, with years between cases, is unlikely to be due to chance. Thus data from 586 patients with poliomyelitis in Massachusetts and Vermont showed that 157 had one or more relatives other than members of their actual household who had had the disease. On investigation of the blood-relatives of 100 normal persons only 5 were found to have had poliomyelitis. The fact that a history of two attacks of poliomyelitis in the same person is by no means rare may also point to the fact that a particular genetic constitution favours infection.

Webster (1937) showed that it was possible to build up a strain of mice susceptible to St. Louis encephalitis virus and a strain which was highly resistant, Cross-breeding with resistant and susceptible lines proved that resistance to the virus was inherited on a single-factor basis with resistance dominant over susceptibility. The increase of resistance to St. Louis encephalitis virus did not entail increased resistance to rabies.

In addition there is a growing body of evidence that the virus infection of hens known as lymphomatosis is modified not only by environmental conditions (Hutt *et al.*, 1944; Waters, 1945), but by genetic factors since it is possible to obtain strains of hens with high and low resistance to the infection (Hutt *et al.*, 1941; Hutt *et al.*, 1944; Taylor *et al.*, 1943; Hutt *et al.*, 1945; Waters, 1946). When the filterable sarcoma of hens was first isolated the virus would attack only

* This lasting immunity is often acquired while the child is still passively immunized by the immune bodies in the mother's milk, breast feeding continuing in Africa for two to three years.

Rhode Island Reds : now, after many passages there is scarcely any breed of hen which cannot be infected while the virus has even gained the capacity to infect heterologous species such as the pheasant (Andrewes, 1932). There is considerable evidence to show that genetics plays an important role in the development of mouse leukæmia (MacDowell, 1937), and it has long been suspected that heredity has a role in the genesis of cancer (Little, 1941) ; in the formation of mammary cancer in the mouse it seems, together with age and ovarian hormones, to play an extremely important part (Bittner, 1945).

In the plant world one of the most important practical tasks of the geneticist is the breeding of strains which are resistant to particular viruses. The question is complicated because in some instances as in the case of resistance to tobacco mosaic virus there is more than one factor inducing resistance. Thus the so-called necrosis factor in *Nicotiana* can be isolated from the mosaic factor and both are related to the temperature of the environment (McKinney and Clayton, 1945). Every plant of the potato "King Edward" carries a virus which produces no ill effect but when it is communicated by grafting to the potatoes "Arran Victory" or "Arran Chief" it causes a crippling and incurable disease, paracrinkle (Salaman and Le Pelly, 1930).

AGE.

It is a well-known epidemiological fact that the common exanthemata, measles, rubella, chicken pox, mumps, and warts are more common in childhood than in later years. This age distribution is probably due in most cases to exposure to the virus in youth and to the development of a specific immunity, often in the absence of clinical symptoms. Measles, when it was first introduced into the South Sea Islands attacked all age groups. During the late war West African troops were constantly having outbreaks of varicella which despite close contact did not spread to European soldiers of the same age group.

Nevertheless in animals there is a definite decrease in liability to virus infections with age in the absence of any possible previous exposure to the virus. This was described by Andervont (1929) in the case of baby mice inoculated intraperitoneally with herpes virus. It is demonstrable in rabbits inoculated with Borna disease (Nicolau and Galloway, 1928) and has since been found to hold good in mice for yellow fever (Theiler, 1930), vesicular stomatitis (Olitsky *et al.*, 1936) rabies (Casals, 1940) encephalomyelitis of mice (Theiler and Gard, 1940), eastern equine encephalomyelitis (Morgan, 1941), western equine encephalomyelitis (Olitsky *et al.*, 1936), and MM poliomyelitis in mice and guinea pigs (Jungeblut *et al.*, 1942 ; Jungeblut, 1947).

It must be noted that the increase in resistance with age is shown only against subcutaneous, intravenous and intraperitoneal inoculation and against intranasal instillation of the virus : if the injection is made intracerebrally there is no difference in susceptibility. It is possible that in the very young mouse the permeability of the blood-brain barrier is greater than in the adult : the capillaries of the central nervous system are supported, according to Broman (1941), by a glial membrane and in the very young this supporting membrane is less developed. In the adult the brain capillaries are impermeable to

Crawley (1948) showed that at temperatures above 37° C. while the virus titre is not increased deaths are accelerated. Lesions in the brains of mice infected with a standard dose of St. Louis encephalitis tend to be at a minimum during the warm season of the year or when high environmental temperatures are maintained: the lesions tend to intensify at lower temperatures. The incidence of deaths after uniform doses did not differ significantly in mice held at 95°, 70° and 42° F. though deaths were a little earlier at a higher environmental temperature (Lillie *et al.*, 1937). Castaneda (1937) found that typhus rickettsiæ would grow profusely in the peritoneal cavity of guinea pigs only when the body temperature was kept below 38° C., Strains of rickettsiæ are easier to maintain in guinea pigs in winter than in summer and incidentally far easier to passage in cold climates than in the tropics.

In the case of bacterial infections there is experimental evidence to show that a fall in temperature causes a reduction in resistance. Pasteur, Joubert, and Chamberland (1878) demonstrated that hens became susceptible to anthrax after standing in ice-cold water and Findlay (1923) and Werkman (1923) found that pigeons died from pneumococcal septicæmia when their body temperature was reduced below 40° C. These effects are probably due to interference with phagocytosis for both opsonization and phagocytosis are found to be increased by a rise of temperature (Harmon *et al.*, 1946).

In plants it has been shown that it is only at certain temperatures that genes regulating the expression of tobacco mosaic disease can be detected. This has been demonstrated for *Capsicum* by McKinney and Hills (1941) and for various species of *Nicotiana* and tobacco hybrids by McKinney and Clayton (1945).

Solar radiation causes the lesions of blue tongue in sheep to be greatly enhanced, more especially if the sheep are photosensitized (Neitz and Riermerschmid, 1944).

Temperature may influence virus infections by altering the bacterial flora of the nose or intestine. Armstrong (1938) pointed out that the sterile Seitz-filtered medium in which nasal bacteria have grown has an inhibitory action on influenza A virus. The inhibitory substance is probably a polysaccharide.

VIRUS INFECTIONS AND NUTRITIONAL FACTORS.

It has long been recognized that nutrition influences virus infections, but not necessarily in the same way as it does helminthic or bacterial infections.

Underwood (1789) noted that infantile paralysis picked out the healthiest and best-fed children while Heine (1860) came to a similar conclusion. More recently De Rudder and Petersen (1938) suggested that the reason why athletic school boys are specially liable to develop severe paralysis is that dehydration of the tissues assists in the dissemination of the virus. Undoubtedly exercise during the incubation or preparalytic period increases the severity of the paralysis (Tucker, 1941; Russell, 1947).

Clinical impressions suggest that in other diseases due to viruses the well-nourished are more likely than the under-fed to be more severely attacked. In the world pandemic of influenza in 1918-19 young robust adults appeared to be the most severely affected. In the tropics, foot-and-mouth disease among poorly

fed cattle is not a particularly severe infection but viruses isolated in the tropics when tested on well-nourished cattle in Europe cause a fulminating infection. Olitsky *et al.* (1928) found that in the laboratory deficiently fed guinea pigs showed increased resistance to foot-and-mouth disease. Rous (1911) noted that unhealthy hens are less susceptible to sarcoma virus than are healthy ones. Rivers (1939) and Sprunt (1942a) showed that if rabbits are deprived of food the skin lesions due to the vaccinia virus are fewer in number and smaller in size than those seen in well-fed rabbits. A full supply of water also seems to reduce the size and number of the vaccinal lesions, possibly by producing œdema of the skin and thus decreasing the concentration of the spreading factor (Sprunt, 1942; Taylor and Sprunt, 1943). Local œdema and inflammation of the skin has an effect on the development of herpes (Olitsky and Schlesinger, 1941). More especially in mice a number of experiments have now been carried out to determine the effect of specific dietary deficiencies on the course of virus infections. Mice given a generally restricted diet are according to Foster *et al.* (1944a) more resistant to poliomyelitis virus as shown by a delay in the time of onset of paralysis and death. Rasmussen *et al.* (1944a) obtained similar results. Restriction of fat or carbohydrate in the diet had no effect on infections due to the virus of mouse encephalomyelitis, nor had high or low protein intakes (Kearney *et al.*, 1948a).

The results of deprivation of specific substances in the diet on virus infections are shown in the table on page 28.

The most striking results of increased resistance are those associated with aneurin deficiency which is apparently effective in increasing resistance against poliomyelitis, mouse meningoencephalitis and herpes. Waisman *et al.* (1945), for instance, obtained complete protection of mice against poliomyelitis virus when the mice were first given 10 μ g. of aneurin for some days followed, when deficiency occurred, by 30 μ g. per 100 g. of body weight. Toomey *et al.* (1944) observed fewer paralyses in aneurin-deficient mice inoculated with poliomyelitis but nevertheless there was a high death rate. In cotton rats inoculated by a number of extracerebral routes as well as intracerebrally with the Lansing strain of poliomyelitis virus Weaver (1945) found no change in susceptibility as a result of deficiency of vitamin B complex. This was also true of the experiments by Clark *et al.* (1945) with the rhesus monkey on a deficient aneurin diet. Deficiency of aneurin in the diet appears to be more effective than a general dietary restriction in increasing resistance to poliomyelitis. In mice infected with Western equine encephalomyelitis virus Kearney *et al.* (1948b) observed that after severe signs of aneurin deficiency had developed there was very little paralysis and the mice showed only atony, tremors, and difficulty in maintaining balance. The time of death was slightly delayed as compared with mice on an optimum diet but it was earlier than in uninoculated mice or in those infected intracerebrally with a suspension of normal brain. Mice on the deficient diet inoculated before the deficiency was marked showed characteristic signs of poliomyelitis infection.

In avian encephalomyelitis the role of aneurin deficiency was less clear cut, the susceptibility of chickens depending on their age, the previous nutritional state, as well as the amount of aneurin fed (Cooperman *et al.*, 1946).

Not every virus necessarily reacts to the same deficiency in the same way. Pantothenic acid deficiency has no effect on resistance to poliomyelitis in the mouse but it increases slightly the resistance to mouse meningoencephalitis virus. Riboflavin deficiency, on the other hand, does not influence resistance to mouse meningoencephalitis but does increase resistance to poliomyelitis. The effect of tryptophan deficiency on the resistance of the mouse to encephalomyelitis is peculiar for though resistance is increased and the disease is often rendered

TABLE I.—Nutritional Factors and Resistance to Virus Infections.

Deficiency.	Virus.	Animal species.	Effect on Resistance.	Authority.
Aneurin	Poliomyelitis	Mouse	Increased	Foster <i>et al.</i> (1944b)
"	"	"	"	Toomey <i>et al.</i> (1944)
"	"	"	"	Waisman <i>et al.</i> (1945)
"	"	"	"	Rasmussen <i>et al.</i> (1944a)
"	"	Rhesus monkey	No change	Clark <i>et al.</i> (1945)
"	"	Cotton rat	"	Weaver (1945)
Vitamin A	"	"	Decreased	Weaver (1946)
Pantothenic acid	"	Mouse	No change	Lichstein <i>et al.</i> (1944)
Riboflavin	"	"	Slightly increased	Rasmussen <i>et al.</i> (1944b)
Folic acid	"	Rhesus monkey	Increased	Pinkerton (1942)
Lysine	"	Mouse	No change	Lichstein <i>et al.</i> (1946b)
Tryptophan	"	"	Delayed onset only	Jones <i>et al.</i> (1947)
Potassium	"	"	No change	Jones <i>et al.</i> (1947)
Pyridoxine	"	"	"	Lichstein <i>et al.</i> (1945)
Inositol	"	"	"	Lichstein <i>et al.</i> (1945)
Biotin	"	"	"	Lichstein <i>et al.</i> (1945)
Pantothenic acid	Mouse encephalomyelitis	"	Slightly increased	Lichstein <i>et al.</i> (1944)
Riboflavin	"	"	No change	Rasmussen <i>et al.</i> (1944b)
Potassium	"	"	Increased	Lichstein <i>et al.</i> (1946a)
Tryptophan	"	"	"	Kearney <i>et al.</i> (1948a)
Aneurin	"	"	"	Waisman <i>et al.</i> (1945)
"	"	"	"	Kearney <i>et al.</i> (1948a)
p-Aminobenzoic acid	"	"	No change	Kearney <i>et al.</i> (1948a)
Phosphorus	"	"	Increased	Lichstein <i>et al.</i> (1946a)
Calcium	"	"	No change	Lichstein <i>et al.</i> (1946a)
Sodium	"	"	"	Lichstein <i>et al.</i> (1946a)
Magnesium	"	"	"	Lichstein <i>et al.</i> (1946a)
Chlorine	"	"	"	Lichstein <i>et al.</i> (1946a)
Aneurin	Herpes	"	Increased	Cutting (1947); Cutting <i>et al.</i> (1947)
"	Avian encephalomyelitis	Chicken	No change	Cooperman <i>et al.</i> (1946)
"	Western equine encephalomyelitis	Mouse	Increased	Kearney <i>et al.</i> (1948b)
Folic acid	Rous sarcoma	Chicken	Increased	Little <i>et al.</i> (1948)

inapparent, the virus undoubtedly multiplies readily in the central nervous system and in addition virus action apparently precipitates the signs of tryptophan deficiency. So little is yet known of the metabolic requirements of viruses that it is not yet possible to give any real explanation of the reasons why depletion of certain factors in the diet should increase the resistance of the host against some virus infections. There is, however, suggestive evidence that in the case of the lymphogranuloma-psittacosis group of viruses the sulphonamides owe

their effectiveness to competition with *p*-aminobenzoic acid which thus becomes an essential virus metabolite (Findlay, 1948a). In the case of aneurin deficiency also there would be competition between the actively multiplying virus and the host cells which it parasitized, aneurin being regarded as another essential metabolite for both virus and host cell. Such a concept is in agreement with the curious fact discovered by Howe and Bodian (1941) that if the axones of nerve cells in a monkey are cut a fortnight before infection with poliomyelitis the regenerating neurones are exempt from infection. The nucleic acid metabolism of regenerating neurones is greatly altered and since viruses contain nucleoproteins virus multiplication must involve an increase in virus nucleoprotein either by a new synthesis or by transformation of host materials. The earliest signs of virus activity are seen histologically in the neurone in the dissolution of Nissl substance but, as Bodian (1947) points out, mere depletion of nucleic acid cannot account for resistance to virus infection since the greatest degree of resistance occurs in regenerating cells when reconstruction of Nissl bodies is well under way. It can, however, be postulated that an important factor in the phenomenon of induced virus resistance in these cells is a disturbance in the molecular arrangements of the complex nucleoprotein aggregate known as the Nissl body. With greater competition on the part of the regenerating cell for materials with which to form nucleoprotein the virus is unable to obtain sufficient supplies to enable it to multiply within the cell.

Imbalance in the diet may also alter the susceptibility to viruses. Sprunt (1948) showed that when mice were fed on a low protein diet and were infected for 14 days with 15 mgm. methionine before being inoculated intranasally with swine influenza virus they became more susceptible than if kept on an inadequate or adequate protein diet.

Pearce and Lange (1947) found that normally there were few cardiac lesions in rabbits inoculated intratesticularly with virus III: when, however, the rabbits were given injections of adrenaline, pitressin or barium chloride, in order to produce cardiac anoxia, the lesions in the heart were more pronounced. Sprunt (1942b) similarly found that the resistance of the rabbit to vaccinia is increased by giving subcutaneous injections of methionine: two other substances with an easily transferable methyl group, choline and to a less extent betaine behave similarly.

Sellards and McCann (1944) found that choline added to the diet of monkeys inoculated with the viscerotropic strain of yellow-fever virus increased their resistance. Findlay (1948b) correlated the deficient diet of the West African with his greater liability not to infection but to death from infective hepatitis. Dramatic recoveries from infective hepatitis have been attributed to the intravenous injection of casein hydrolysate.

The vascular supply and the presence of regenerating epithelial cells are apparently related to resistance against certain virus infections of the skin. Thus Teague and Goodpasture (1923) found that when the skin of the guinea-pig was painted with tar the virus of herpes produced extensive lesions like those of zoster. Kidd and Rous (1938) and Rous and Kidd (1938) showed that if the rabbit's skin were first painted with tar for four weeks inoculation of the Shope papilloma produced luxuriant papillomata and carcinomata.

Friedewald (1942) used 0·3 p.c. methyleholanthrene four or five times at intervals of two days before inoculation and obtained similar results. Alteration in vascular supply associated with the liberation of histamine and other vasodilators is probably the explanation of the occurrence of zoster after trauma or after neoplastic invasion of the nerve root ganglia (Klauder, 1947).

Myocardial infarction, possibly through antidromic nervous stimulation may precipitate an attack of zoster (Shapiro *et al.*, 1947).

The relationship of ascorbic acid to infection with poliomyelitis is still in some doubt. Jungeblut (1939) claimed that ascorbic acid had a curative action in monkeys suffering from poliomyelitis but this could not be confirmed by Sabin (1939). Heaslip (1938) in Australia found that on the average vitamin C stores were less in children with poliomyelitis than in controls but the administration of large doses of vitamin C had no effect on the clinical course of the infection. *In vitro* ascorbic acid undoubtedly has a virucidal action on poliomyelitis (Jungeblut, 1935b), vaccinia (Kligler and Bernkopf, 1937), and influenza A viruses (Knight and Stanley, 1944; Klein, 1945). Klein (1945) showed that the virucidal action of ascorbic acid is inhibited by potassium cyanide, presumably because potassium cyanide combines with copper which is essential for the catalytic oxidation of ascorbic acid. Ascorbic acid could scarcely have a therapeutic action *in vivo* because of the presence of catalase in the tissues.

It will thus be seen that the role of nutritional factors in the control of virus infection varies considerably. In some cases excess nutrition appears to favour virus action: in other cases, as in some bacterial infections, lack of essential nutrients decreases resistance and allows more extensive lesions to develop: in other cases deficient nutrition increases resistance and even if it does not inhibit multiplication of the virus renders the infection inapparent.

It is of some interest to note that in a fungus infection of *Narcissus* the addition of growth stimulants to the bulbs renders them more susceptible to the pathogenic action of the basal rot fungus, *Fusarium oxysporium f. narcissi* (Stuart and McClellan, 1943). On the other hand in infections due to bird malaria Seeler and Ott (1944) find that with *Plasmodium lophurae* riboflavin deficiency in chicks decreases infection as measured by the percentage of red cells parasitized but increases susceptibility as measured by mortality rates. Biotin-deficiency in ducks and chicks infected with *P. lophurae* and in ducks infected with *P. cathemerium* increased the degree of parasitism (Trager, 1943). In experimental typhus in the guinea-pig and rat Zinsser *et al.* (1931) showed that a diet deficient in vitamins decreased the resistance to infection: Pinkerton and Bessey (1939) found that the essential factor in reducing resistance was riboflavin. Some bacteriophages cannot lyse bacteria unless specific amino-acids are present in the culture media.

VIRUS INFECTIONS AND HORMONES.

Comparatively few observations have been made on the effects of hormone administration on virus infections. Holtman (1946a) found that mice which had become acclimatized to life at a low temperature were less susceptible to poliomyelitis than those kept at normal temperature. This difference might be due to an altered metabolic rate, interfering with the ability of the virus to

establish itself, thyroxin secretion being greater on exposure to cold. Mice were therefore given thyroid extract, thiouracil, and a thyroactive iodocasein daily, before and after inoculation with poliomyelitis virus (Holtman, 1946b).

No. of mice.	Treatment before inoculation.	Period in days required to effect a 50 p.c. mortality.
20	0.5 mg. thiouracil daily.	5
8	0.5 mg. thyroid extract daily.	11
36	0.5 mg. thyroprotein (thyroactive iodocasein) daily.	14
20	Untreated controls.	7

These results suggest that thyroid activity may be correlated with the decrease of poliomyelitis in cold weather.

It has long been thought on clinical grounds that pregnancy predisposed to poliomyelitis although adequate statistical evidence is lacking (Bradley and Gale, 1948). However, immature castrated female monkeys apparently develop the disease more promptly and more frequently after intranasal instillation than do similar animals prepared by administration of œstrin (diethylstilboestrol). Such monkeys are not resistant to intracerebral inoculation (Aycock, 1937, 1940 ; Aycock and Curley, 1947).

Although pregnant women are not more susceptible to infective hepatitis than others the disease is frequently much more malignant in them and the mortality is frequently high. Treatment of castrate female or normal male and female mice with α -estradiol dipropionate or diethyl stilbestrol enhances resistance against intranasal instillations of poliomyelitis virus (Foley and Aycock, 1945). The milk-borne virus responsible for breast carcinoma in mice is apparently forced into activity by doses of œstrogen (Bittner, 1945 ; Pullinger, 1947).

Murphy (1944) reported that the incidence of leukæmia in female mice of the Rockefeller Institute strain was about twice that in males of the same litters. Castration of females made little change in the incidence of leukæmia but castration of males increased the incidence to that of normal and castrated females. Testosterone given to ovariectomized mice reduced the incidence of leukæmia to that of normal males. The sex difference in susceptibility would thus seem to be due to the inhibitory effect of male sex hormone rather than to a stimulation of ovarian secretion.

In fowls infected with the virus of lymphomatosis Burmester (1945) found that the incidence in females was twice as high as in males of the same population. Marine and Rosen (1940) and Burmester and Nelson (1945) noted that castrated males had a significantly higher incidence of lymphomatosis than normal males of the same stock. Males and capons treated with testosterone had a significantly lower incidence than untreated males and capons. Here again the male hormone would seem to increase resistance to a virus infection.

Jungeblut (1935a) found that pregnant mare serum was capable of inactivating poliomyelitis virus.

VIRUS INTERFERENCE.

The belief that once an individual has become infected with one disease he thereby becomes resistant to another is very old. Montaigne (1580) maintained

that "one ill cureth another" while Thomas Middleton (1604) asserted that "Seriant Carbuncle, one of the plague's chiefe officers, dare not venture within three yardes of an Harlot because Mounseer Dry-Bones the French-Man is a lodger before him." In the eighteenth century, it was noted that children suffering from florid secondary yaws invariably developed very mild attacks of variola (Quier, 1780). Winterbottom (1803) recorded that in Sierra Leone some African tribes were accustomed to treat chronic ringworm by inoculating the affected area of skin with herpes. Archer (1809) reported that vaccination in children benefited whooping cough and in mice Dalldorf *et al.* (1947a, b) showed that under certain circumstances the injection of vaccinia virus intracerebrally protects against the development of infection due to *Hæmophilus pertussis*.

Hodge (1948) has drawn attention to the fact that measles may abort whooping cough.

The first example of one virus infection protecting against another was recorded by Jenner (1804) who found that vaccinia gave only a very feeble reaction on skin involved in a herpes eruption.

Modern interest in what has been termed "virus interference" was first aroused by plant pathologists. McKinney (1929) observed that common tobacco mosaic virus interfered with the growth of yellow tobacco mosaic. Thung (1931) also worked with strains of tobacco mosaic virus while Salaman (1933) reported similar interference between strains of potato virus. A number of other examples of plant virus interference have now been recorded: the available information is summarized by Price (1940). The same phenomenon occurs in association with animal viruses. Some strains of herpes virus placed on the scarified cornea of the rabbit migrate along the optic nerve to the brain causing an encephalitis, other strains remain localized in the cornea. Magrassi (1935a, b) showed that if the rabbit's cornea were inoculated with a non-encephalitogenic strain of herpes the progress of an encephalitogenic strain to the brain is blocked. Hoskins (1935) found that in monkeys prior inoculation of the relatively non-pathogenic neurotropic strain of yellow fever protected against subsequent infection by the more virulent viscerotropic strain. This was confirmed by Findlay and MacCallum (1937) who also found that Rift Valley fever would protect monkeys against the viscerotropic yellow fever virus, while the neurotropic strain of yellow fever virus would protect mice against the highly pathogenic Rift Valley fever virus. These results were confirmed by De Assumpção (1944) in rhesus monkeys and camodongos. Dalldorf, Douglass and Robinson (1938) showed that lymphocytic choriomeningitis virus will protect monkeys against poliomyelitis virus. These observations proved that the phenomenon of virus interference is quite distinct from that of specific immunity for the interfering virus need not be related antigenically to the virus with which it interferes. The hypothesis put forward by Findlay and MacCallum (1937) was that both viruses compete for the same chemical groups in susceptible cells. Examples of interference between animal viruses are recorded in Table II. The phenomenon of virus interferences is also seen with bacteriophages (White, 1937) and here interference can be viewed by the electron microscope (Dëlbrück and Luria, 1942). Bacteriophage inactivated by ultra-violet light can interfere with the action of live virus while heated and killed animal viruses in some cases inter-

fere with living viruses. Immunization against rabies with a killed or attenuated fixed virus originally introduced by Pasteur would seem to be an instance of virus interference (Rhodes, 1947) while Green and Stulberg (1946) have deliberately made use of virus interference to immunize against dog distemper.

Inactivation of a mouse-adapted MM poliomyelitis virus by exposure to ultra-violet light or to sodium hypochlorite (3150 parts per million) does not

TABLE II.—Interference between Animal Viruses.

Virus Inhibited.	Interfering Virus.	Reference.
1. Antigenically related Viruses. (a) Interference <i>in vivo</i> .		
Herpes simplex (encephalitic strain)	Herpes simplex (non-encephalitic strain)	Magrassi (1935a, b)
Yellow fever (viscerotropic strain)	Yellow fever (neurotropic strain)	Hoskins (1935); Findlay and MacCallum (1937)
Rinder pest	Rinder pest (goat-adapted strain)	Pfaff (1938)
Rinder pest	Rinder pest (Egg-adapted strain)	Jenkins and Shope (1946)
Dog-distemper	Dog-distemper (Ferret-adapted strain)	Green (1946)
Vaccinia (neurotropic strain)	Vaccinia (commercial)	Dalldorf <i>et al.</i> (1947a, b)
Psittacosis (6 BC strain)	Psittacosis (SF strain)	Golub (1947)
Poliomyelitis (Monkey strain)	Poliomyelitis (Mouse-adapted MM strain)	Jungeblut (1945) Jungeblut and Sanders (1940), (1942)
(b) Interference in Tissue Culture or in the Chick Embryo.		
Influenza A (pneumotropic mouse strain)	Influenza A (neurotropic mouse strain)	Andrewes (1942)
Influenza A	Influenza A (inactivated by ultra-violet light)	Henle and Henle (1943, 1944); von Magus (1947)
Influenza B	Influenza B (inactivated by ultra-violet light)	Henle and Henle (1943, 1944)
Ectromelia	Ectromelia (inactivated by ultra-violet light)	Andrewes and Elford (1947)
Blue tongue virus of sheep	Blue-tongue (killed)	Alexander (1947)
2. Antigenically distinct Viruses. (a) Interference <i>in vivo</i> .		
Vaccinia	Herpes	Jenner (1804)
Shope papilloma virus	Herpes	Syverson and Berry (1947)
Yellow fever (viscerotropic strain)	Rift Valley fever	Findlay and MacCallum (1937)
Rift Valley fever	Yellow fever (neurotropic strain)	Findlay and MacCallum (1937); de Assumpção (1944)
Poliomyelitis (Monkey strain)	Lymphocytic choriomeningitis	Dalldorf <i>et al.</i> (1938); Dalldorf (1939)
Shope fibroma	Virus III	Andrewes (1940)
Western equine encephalomyelitis	Mouse meningo-encephalitis	Schlesinger <i>et al.</i> (1943)
Eastern equine encephalomyelitis	Western equine encephalomyelitis	Schlesinger <i>et al.</i> (1943, 1944)
Shope papilloma	Sheep dermatitis	Selbie (1946)
Sheep dermatitis	Shope papilloma	Selbie (1946)
Vesicular stomatitis	Western equine encephalomyelitis	Schlesinger <i>et al.</i> (1943, 1944)
Ectromelia	Vaccinia	Andrewes and Elford (1947)
Psittacosis (6 BC strain)	Mouse meningopneumonitis (Cal. 10 strain)	Golub (1947)

TABLE II.—Interference between Animal Viruses.

Virus Inhibited.	Interfering Virus.	Reference.
(b) Interference in Tissue Culture or in the Chick Embryo.		
Western equine encephalomyelitis (Lederle strain)	Influenza B	Vilches and Hirst (1947)
Eastern equine encephalomyelitis	Influenza A	Vilches and Hirst (1947)
Bwamba Forest virus	Influenza A	Vilches and Hirst (1947)
St. Louis encephalitis	Western equine encephalomyelitis	Duffy (1944)
St. Louis encephalitis	Influenza A	Vilches and Hirst (1947)
Eastern equine encephalomyelitis	Influenza B	Vilches and Hirst (1947)
Bwamba Forest virus	Influenza B	Vilches and Hirst (1947)
St. Louis encephalitis	Influenza B	Vilches and Hirst (1947)
Western equine encephalomyelitis (Lederle Strain)	Mumps	Vilches and Hirst (1947)
Western equine encephalomyelitis (Lederle Strain)	Newcastle disease	Vilches and Hirst (1947)
Influenza A	West Nile virus	Lennette and Koprowski (1946)
Influenza A	Yellow fever (17 DD High Strain)	Lennette and Koprowski (1946)
Influenza A	Influenza B	Ziegler <i>et al.</i> (1944a, b)
Influenza A	Swine influenza	Ziegler <i>et al.</i> (1944a, b)
Influenza B	Influenza A	Ziegler <i>et al.</i> (1944a, b)
Influenza B	Swine influenza	Ziegler <i>et al.</i> (1944a, b)
Swine influenza	Influenza A	Ziologer <i>et al.</i> (1944a, b); Sigel (1947)
Swine influenza	Influenza B	Ziegler <i>et al.</i> (1944a, b)
Western equine encephalomyelitis	Influenza A (alive and inactivated by ultra-violet light)	Vilches and Hirst (1947)
Venezuelan equine encephalomyelitis	Yellow fever (17 DD High Strain)	Lennette and Koprowski (1946)
Venezuelan equine encephalomyelitis	West Nile virus	Lennette and Koprowski (1946)
West Nile virus	Yellow fever (17 DD High Strain)	Lennette and Koprowski (1946)
Psittacosis (6 BC strain)	Mouse meningo-pneumonitis (Cal. 10 strain: alive and heated at 56° C. for 2 or 3 minutes)	Golub (1947)
Mouse meningopneumonitis	Psittacosis (6 BC strain) (inactivated by U.V.L.)	Golub (1947)

destroy the capacity of the virus particles to interfere with active poliomyelitis virus (Jungeblut, 1948).

The fact that dead viruses are able to interfere with the pathogenic action of other viruses is not surprising since it was shown by Ledingham (1926, 1927) that the intradermal injection of Indian ink in the rabbit renders the injected area of skin insusceptible to vaccinia virus. There is one other way in which one disease may influence resistance to another although comparatively few experimental investigations have been made. Zilber and Baidakova (1945) have found that after subclinical doses of influenza virus the permeability of the lung tissue to typhoid immune serum is greatly increased. It is thus possible that the permeability of the lining of the lower respiratory tract for antibodies may be influenced by non-specific infections (Jones, 1924).

The opposite phenomenon where one virus favours the growth of another is

not unknown. Tumour cells favour the survival and growth of a number of viruses and the same is true of the Shope papilloma in rabbits (Syverton and Berry, 1947). In addition the presence of influenza virus and of mouse pneumonia virus stimulates the growth of tubercle bacilli in the lungs of mice (Volhert *et al.*, 1947). The association of bacterial infections as secondary invaders to viruses is of course well known in human influenza, dog distemper and various feline infections.

SPECIFIC RECEPTORS AND RESISTANCE.

Fresh evidence has been thrown on the mechanism by which a cell is refractory to the attacks of viruses by the discovery of specific receptor groups on the surface of certain cells. Hirst (1942a, b) showed that the red cells of hens are agglutinated when mixed with a suspension of influenza virus A or B. This agglutination is inhibited by immune serum. Later Hirst (1943) found that the cells lining the bronchi of mice also possess receptors for influenza virus. Investigations have shown that some other viruses, mumps, Newcastle disease and infectious mononucleosis are able to agglutinate the red cells of certain species. Once red cells have been agglutinated by a virus they cannot be further agglutinated by another virus but the virus particles can be eluted from the red cells to which they adhere and such red cells are then no longer agglutinable. In addition the specific receptor groups on the cell surfaces can be destroyed by certain enzymes such as one of those produced by *Vibrio cholerae*, and *Clostridium welchii* (Burnet *et al.*, 1946).

The specific substrate is presumably a carbohydrate since it is destroyed by periodate while Green and Woolley (1947) have shown that certain complex carbohydrates such as apple pectin, citrus pectin, blood group A substance, gum acacia and flax-seed mucilage inhibit red cell agglutination by influenza viruses. Horsfall and McCarty (1947) similarly find that the course of mouse pneumonitis virus infection is modified by bacterial polysaccharides.

Thus a cell may be resistant to the attack of a virus because:—

- (1) The cell does not possess specific receptor groups.
- (2) The receptor groups though originally present have been—
 - (a) occupied by other chemical groups ;
 - (b) destroyed by enzymes ;
 - (c) removed by elution.

PSEUDO-VIRUCIDAL BODIES.

There is some evidence that the sera of certain animals and men may contain substances which have the power of inactivating certain viruses, although such animals or men have not previously been exposed to the particular viruses. The two virus infections in which this condition is specially liable to occur are poliomyelitis and yellow fever. In yellow fever it is known that the sera of certain cows, goats, horses, and sheep neutralize the virus when tested by the mouse protection test (Findlay *et al.*, 1936 ; MacCallum and Findlay (1937) ;

Saleun, 1939 ; Smith, 1940 ; Findlay, 1941) : some of the cows which protected against yellow fever came from England, others from the slopes of the Himalayas. In addition to cows, sheep and other domestic animals the sera of certain rodents has been found both in Africa and in South America (Findlay and Cockburn, 1943 ; Laemmert *et al.*, 1946) to neutralize the yellow fever virus. Of 1794 rodents tested in one endemic area in Brazil 449, or 25 p.c., gave positive reactions, but contrary to what would be expected of an acquired specific immunity the proportion of positive reactors does not increase with age.

A further complication, so far as the epidemiology of yellow fever is concerned, is the finding of virucidal bodies in the blood of certain birds in Brazil (Laemmert *et al.*, 1946), Central Africa (Saleun, 1939), West Africa (Findlay and Cockburn, 1943) and in the Zambesi Valley where human positive sera were obtained but no primates were found with positive blood sera (South African Institute for Medical Research, 1947). Of 114 birds examined in Brazil, the sera of 3 inactivated the virus of yellow fever : in South Africa 2 of 6 and in West Africa 4 of 40, while in addition 2 of 8 domestic Guinea fowl have been found to give a positive mouse protection test. Laemmert and Moussatché (1943) working with laboratory strains of yellow fever virus have found that yellow fever virus will circulate in the blood stream of certain birds for several days after it has been injected by the syringe but attempts to infect birds by mosquito bites have not been very successful.

In man there have been found on rare occasions bodies in the serum which neutralize the yellow fever virus by the mouse protection test. Sawyer *et al.* (1937) reported that the serum of a Tamil from the Madras Presidency protected mice against yellow fever virus in a dilution of 1 in 10. Serum from an adult male in Toronto, Canada also inactivated the virus. The most striking instance, however, is that reported by Das Gupta and Sen Gupta (1946). A Bengali girl of eleven years had a severe illness with jaundice and black vomit : her serum gave no positive tests for leptospiral jaundice, but on the 62nd and 116th days after onset protected mice against yellow fever virus in a dilution of 1 in 64 ; a sample of serum taken 387 days after the onset of illness showed no protective power at all. The virucidal bodies were not therefore those of yellow fever. Elucidation of the origin and nature of these virucidal bodies which occur in a small percentage of specimens of sera from certain species of animals and birds is obviously a matter of considerable importance not only for the epidemiology of yellow fever but for the study of resistance to virus infections in general.

Very much the same problem exists in regard to poliomyelitis virucidal bodies present in the blood serum of a very high percentage of human beings. It is still uncertain whether these bodies are true immune bodies in the sense that they have developed as a result of previous exposure to the virus or whether they play any part in protecting the central nervous system from invasion by the virus of poliomyelitis (Hudson *et al.*, 1936). In tropical countries such as for instance West Africa poliomyelitis does not occur in epidemic form among the indigenous inhabitants but is endemic among children (Findlay *et al.*, 1946) ; all ages, however, from 1 year up, show the presence of virucidal bodies against the M.E.F. I strain (Olitsky and Findlay, 1946). Non-indigenous adults, however, are susceptible to local strains of virus. In Western Europe there is now a

tendency for older age groups to be more often attacked, but the shift in incidence to these older age groups is by no means uniform. It is suggested on the one hand that the change in age distribution is due to increased sanitation and that this is one of the prices which must be paid for efficient sanitary disposal. On the other hand Son Bertenius (1947) as a result of experience in Scandinavia believes that immunization by subclinical infection probably plays no part in determining the incidence of poliomyelitis. A "predisposition" of the individual probably produced by social factors is regarded as a necessary antecedent to acquiring poliomyelitis. The reason why poliomyelitis should during the last half century have given rise to world wide epidemics is still an unsolved mystery. In poliomyelitis the status of these virucidal bodies is till further complicated by the fact that an attack of poliomyelitis does not necessarily produce any increase in virucidal bodies in the blood. Thus Hammon *et al.* (1947) found that of 102 patients whose sera were collected when the disease was first suspected and again 15 to 30 days later, 84 showed no significant change, 8 showed a decrease, and only 10 showed an increase in the titre of virucidal bodies.

Virucidal bodies found in the nasal secretions of man are specific for poliomyelitis (Lansing strain); they do not inhibit St. Louis or Western equine encephalomyelitis virus (Bell, 1948).

Virucidal bodies against poliomyelitis virus have also been found in the bloods of animals. Gordon (1945) tested sera from 37 dogs and found 3 positive. Hammon *et al.* (1947) found 10 of 19 dogs positive, 10 of 15 goats, 3 of 10 sheep, 3 of 4 pigs and 0 of 10 cats. Among 55 domestic cows 43 gave positive sera, but of 18 from the Nevada desert none had positive sera, similarly of 48 domestic horses 67 p.c. were positive but of 15 desert horses only one. Of 30 wild animals (chipmunk, coyote, ground squirrel, mouse, jack and cotton tail rabbits), none were positive. Similarly of 63 sera from domestic birds 19 gave positive tests but of 26 wild birds only 5. The true significance of these tests is unknown.

It is not without interest that Flexner and Amoss (1920) found that certain rhesus monkeys could not be inoculated with poliomyelitis intranasally although these monkeys had no antiviral bodies in the serum. Similarly Amoss and Taylor (1917) and Howitt (1937) found that the nasal washings of certain human beings neutralized poliomyelitis virus: this power was not, however, correlated with antiviral bodies in the serum.

DISCUSSION.

At first sight the various factors which, apparently, influence the resistance of the body to virus infections would seem to have little in common. If, however, they are related to the ecology of viruses the majority are seen to involve certain stages in the development of virus infections. Non-specific resistance may in fact be looked upon as involving—

- (1) The portal of entry of the virus into the body.
- (2) The receptor mechanisms of the susceptible cell.
- (3) The food supply of the virus within the susceptible cell.

The virus may not be able to gain a foothold in the tissues because of the condition of the cells at the portal of entry. Thus oestrogens, it is suggested,

affect the cells of the olfactory mucosa so that the poliomyelitis virus cannot gain entrance. A similar idea underlay the attempts, now abandoned, to treat the nasal mucosa with various chemicals such as picric acid, alum or zinc salts as a prophylactic against poliomyelitis. External factors may prevent the virus getting into the body. Thus African soldiers were well aware that if they exposed their recently vaccinated arms to the hot tropical sun they would fail to develop vaccinal lesions, a finding experimentally proved by Lacassagne (1937). The frequency with which poliomyelitis follows tonsillectomy suggests that the tonsils offer a barrier to the virus, this barrier being destroyed by the local trauma. Statistical evidence is given by Glover (1948). Non-specific resistance may be taken one step further back for there are certain persons who appear to be antipathetic to virus-carrying arthropods, mosquitoes, fleas, lice, and ticks. Mr. Pepys, it will be remembered, was one of those who was not appreciated by fleas, for when sharing a bed with Doctor Clerke "at Wiard's the chyrurgeon's in Portsmouth" on April 23, 1662, he remained, unlike his medical companion, unbitten (Pepys, 1893). In laboratories where louse colonies are fed on human hosts it is recognized that after a time the lice, if fed on the same host, tend to die, presumably because they have exhausted some essential food factor in their host's blood or because he has developed some substance which is toxic to them. Brennan (1947) has recorded the case of a white American whose sweat was so lethal to ticks that when they tried to feed on him they fell off dead.

The receptor mechanism of the cell may be damaged or may be occupied by some other substance, a virus or a chemical substance, as in virus interference or by some enzyme present in the blood. The cell is then refractory to virus invasion.

The food supply of the virus may be interfered with. Changes in the vascular supply to the affected part may inhibit the multiplication of the virus. Thus the action of X-rays on warts or on vaccinal lesions is almost certainly dependent on the effect on the vascular supply to the infected cells. The beneficial effect of pituitrin injections on the lesions of zoster probably has a similar explanation as does the curing of warts by suggestion, of which there is now ample evidence to support the folk belief that certain persons possess the power of "charming" warts (Nini, 1923; Bloch, 1927; Sulzberger and Wolf, 1934; Rulison, 1942; Vollmer, 1946). The effect of a high temperature in precipitating an attack of herpes is again most probably due to an increased blood supply to the ganglion cells in which the herpes virus is lying quiescent. Ultra-violet rays by liberating vasodilator substances increase the blood supply and cause increased permeability of capillaries, thus tending to localize virus. The effect of anaphylactic shock in converting a latent herpes into an active encephalitis may also be due to changes in the vascular supply (Good and Campbell, 1948).

Hyperplastic epithelium has been shown by Cowdry (1947) to differ from normal epithelium since it contains less calcium, iron, lipid, and biotin but more magnesium, pyridoxine, and water.

Finally, the effects of deficient nutrition may decrease the powers of proliferation of the virus by cutting off the supply of essential metabolites. If non-specific resistance is built up by a number of separate mechanisms it is doubtful whether it can be legitimately correlated with such a condition as the oxygen

uptake per square metre of body surface during maximal effort, as suggested by Locke (1937), who attempts to relate the liability to the common cold to oxygen uptake.

A much fuller investigation of these non-specific factors in resistance is, however, essential to an understanding of the reasons why one individual becomes infected by a virus and another escapes or why the incubation period varies so greatly in different individuals. In 1942 for instance, according to Horstmann and Paul (1947), 5000 soldiers were inoculated with the same batch and the same dose of icterogenic yellow fever vaccine. Of these 5000 men 1004 developed hepatitis, the incubation period varying from 63 to 137 days. Such a wide variation can hardly be explained on the basis of a specific immunity.

The interplay of virus and host and hence the signs and symptoms of disease may be looked upon as depending on the following factors :—

- (1) The ability of the parasite to penetrate the tissues of the host.
- (2) The transportation of the virus from the portal of entry until it comes in contact with susceptible cells.
- (3) Adsorption of virus on the surface of susceptible cells.
- (4) Penetration of virus into susceptible cells.
- (5) The presence in susceptible cells of sufficient metabolites to allow for the growth and multiplication of the virus.
- (6) The ability of the parasitized host cell to maintain its normal metabolism and its integrity and to support the growth of intracellular virus particles either by obtaining an excess of particular metabolites or by using alternative metabolic paths.
- (7) The presence of an intercurrent parasite giving rise to interference.
- (8) The presence in the blood of virucidal bodies in the serum as distinct from specific immune bodies.
- (9) The presence of specific immune bodies at the surface of susceptible cells at the time of adsorption of the virus.
- (10) The rate at which specific immune bodies can be mobilized at the surface of susceptible cells.

Studies on non-specific resistance if systematically pursued may well lead to results as important as those which have been gained from the investigation of specific immunity.

Bawden (1948) has recently discussed physiological factors in relation to resistance against plant viruses.

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V.—A METHOD FOR THE PREPARATION OF OVARY MATERIAL FROM LILIACEOUS AND SIMILAR TYPES OF PLANTS.

By FREDERICK C. GRIGG, F.L.S., F.R.M.S.

THREE TEXT FIGURES.

DURING cytological investigations slides are liable to accumulate by the hundred and any method calculated to reduce these numbers is highly desirable. The following is a short account of processes I have employed with much success when dealing with plants possessing ovaries of the tripartite type characteristic of *Lilium* and related genera.

It is usual to cut this type of material transversely and even if cut up to $16-18\mu$ a great number of sections containing no embryo-sacs whatever find their way into the ribbon; even by crowding the sections and using 2×1 -inch covers a great number of slides will result from only one ovary, and the triangular disposition of embryo mother cells (E.M.C.) in this plant is not the most convenient for long and sustained examination of many slides. It is also undesirable to cut sections from this type of material too thick for invariably a layer of tapetal tissue will obscure an important division figure, and even if transparent stains are used such as crystal violet with sections up to 40μ , as recommended by

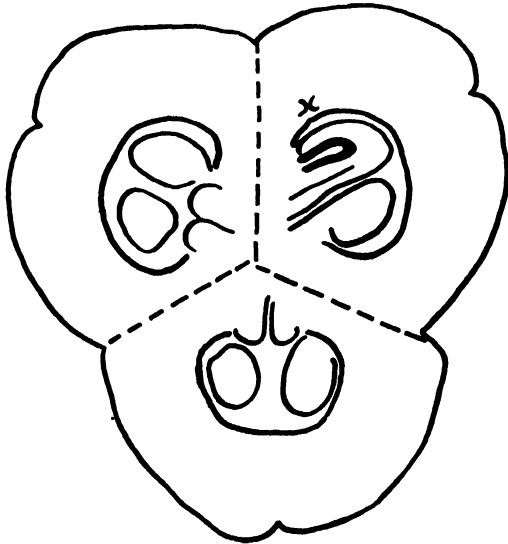


Fig. 1.—Outline drawing of actual transverse section of ovary of *Lilium Henryii*. The dotted lines indicate the longitudinal cuts made in the fresh ovary. It should be noted that only one locus *x* among the entire six contains a central portion of an embryo-sac; this is not invariably the case, but is very common.

Darlington (1942), great difficulty will ensue with tube-length corrections when using a 4 mm. objective of high aperture and performance for photomicrography.

The method I use is as follows : Ovaries are collected and fixative prepared, and then, with a thin sharp knife, the organ is slit downwards almost to the base in three positions, thus dividing it into three parts joined at one end. Fig. 1 will make this quite clear. This has the added advantage of exposing the inner tissue to the fixative and avoiding the rather slippery process of attempting to shave off the outside of the ovary, as suggested by Johansen (1939), to ensure that the fixative will adequately penetrate the ovules. The following processes of dehydration and infiltration are carried out in the usual way. I always use dioxan for dehydration and place the material in a muslin or gauze bag with a small piece of paper bearing the batch number written in pencil, the paper being treated exactly as the material.

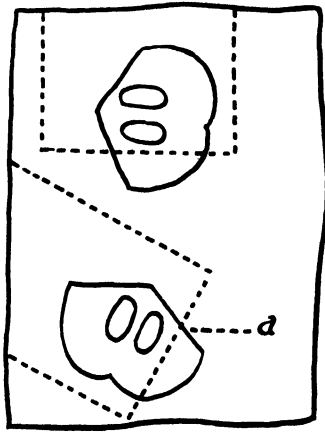


Fig. 2.—Diagram of end of block containing portions of ovary of which two only are figured. The outlines of the resultant microtome blocks are indicated by dotted lines and are as wide as the ovaries are long.

When ready for setting into the paraffin block a small pan is prepared by smearing with glycerine, and a quantity of the wax is run in ; this is allowed to set on the bottom to prevent the material from coming into contact with the edge of the block, and the surface is kept fluid with an electric lamp of 100 watts. The ovaries are taken out of the infiltration vessel and broken or cut into three pieces where they were previously slit, and placed in the casting pan exactly parallel with regard to distance apart and their ends. After setting, the block is divided into portions each containing three pieces of ovary from the same organ, this is trimmed and the ends of the portions of ovary exposed as fig. 2. These are examined under the dissecting microscope and the orientation of the ovules will be plainly visible : it now remains to divide the block into three and trim in a manner calculated to bring these into an accurate line for subsequent sectioning. It is an advantage to cut through the ovary tissue where sectioning is to commence as at fig. 2 (a) so that cutting will engaged every ovule at the same time.

By this method it is possible to mount the sections of an entire ovary of

Lilium on no more than six slides with sections of a reasonable thickness of about 8–12 μ . Indeed, with accurate alignment of the material one is sometimes fortunate to find the principal contents of the six lines of embryo-sacs in no more than 10–12 sections. This can be indicated by the simple process of crossing out the remainder of the sections with glass ink. Sections should be cut transversely to the block rather than longitudinally, as in fig. 3; indeed, a good

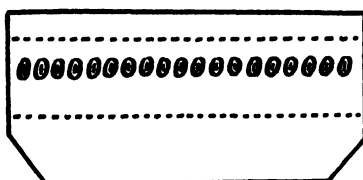


Fig. 3.—Method of trimming block to indicate edges of section if the material is small and difficult to see within the paraffin ribbon. The narrow edge engages the edge of the knife first. Embryo-sacs are depicted in one row and the dotted lines indicate boundaries of ovary tissue.

sharp knife set at a proper angle will cut ribbons from a very narrow block and effect much space saving. Each section will contain about thirty E.M.Cs., and, most valuable of all, as it is the case that the lower cells are progressively later in development than those near the stigma, an instructive sequence of growth changes is secured of a subject which for long eluded the attentions of cytologists. If due care is taken in the orientation of the sections on the slides it is easy to follow up the two or three sections through one embryo-sac. Recording of data can be by section number and E.M.C. number in the particular section as from the top. In my laboratory these slides are placed in holders like a card-index when set hard, and a slip of paper with data notes inserted behind the slides. A separate card index is kept for locating the slides representing different subjects, cells at various stages and under special preparatory techniques.

Annotations on the reference slips are as follows :—

Subject.	Slide No.	Section No.	E.M.C. No.
Diak. late	589/a/IV	33	10
Met-2 cytoglobules	694/n/II	7	2

SUMMARY.

A method is described for dividing ovary material of *Lilium* species to minimize the number of slides used and conveniently orientate the ovules into longitudinal rows for easy investigation instead of employing the more usual transverse method of sectioning. Advantages obtainable for photomicrography are noted, and methods of tabulating for reference the condition of growth of individual E.M.Cs. are included.

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VI.—NOTE ON THE GENUS *FENESTRELLA* GREVILLE.

By C. C. SWATMAN, F.R.M.S.

TWO PLATES.

ATTENTION has recently been drawn by Voigt (1943) to the importance of small details in the Naviculoid Diatoms. This paper deals with similar problems in the genus *Fenestrella* Grev.

Greville (1863) provided a description and figure of a diatom found on a slide of Barbados earth, which he named *Fenestrella barbadensis*. His description is as follows :—

“Frustules free, disciform, disc with minute radial cellulation, interrupted in the middle by linear bands composed of parallel lines of cellules, each band terminating in a flat ocellus.

“This genus is composed for the reception of a solitary but most curious diatom, the relations of which it is not easy to define. The groundwork of the disc is very much that of *Coscinodiscus*, being composed of radiating lines of cellules, with a marginal row of puncta. But a couple of circular ocelli, at little more than half the radius from the centre, although not conspicuous, are sufficiently evident, and show that we must look for affinities elsewhere. These ocelli are not processes, but definite blank spaces in the cellulation, and have therefore no connection with *Eupodiscus* or *Aulacodiscus*.

“The most remarkable feature in the disc, composed, as it is, of a radiating cellulation, is the absence of a central point, there is neither umbilicus or centre of radiation, the band above described lying like a bar across it.”

Later, Brun (1891) described two more species, *F. convexa* and *F. gloriosa*. Both were found in material from Oamaru, New Zealand, and from Yedo, Japan. *Fenestrella convexa* Brun has also been found in the Barbados earth.

Grunow had meanwhile found in the Jutland deposit a large discoid form which he figured in Van Heurck's Synopsis (1880–5) under the name *Janischia antiqua*. This was transferred by Rattray (1890) to *Coscinodiscus* and renamed by him *C. ludovicianus*.

Chenevière (1934) described some diatoms from a newly discovered Russian deposit at Kamischev, and stated : “This deposit is truly extremely curious, for in it one finds nearly all the diatoms of the Jutland Peninsula situated some thousands of kilometers away, as well as diatoms of the Simbirsk group, equally very distant. As to Jutland, we find in Kamischev, *Janischia antiqua*, generally broken, as well as *Odontropis cristata* and *carinata*, which are typical of the Danish deposits, and the series of *Triceratium* and *Trinacria* of Mors and Für.”

Examination of many spreads of Kamischev material have shown but few specimens of *Janischia antiqua* Grun., but an allied and hitherto undescribed

form is found in which the striation is coarser than in *Janischia antiqua* Grun., and the pair of concentric slits of the latter are reduced to two much shorter slits, rather wider and practically straight. In this form the lines of striæ do not all run to the centre of the valve but two bars of cellules run from the ocelli across the valve to meet in the centre area in an irregular line at right-angles to the line joining the ocelli. Some specimens of *Fenestrella barbadensis* Grev. have been found in the same material and are indistinguishable from Greville's specimen. In the course of examination a number of small projections like a set of teeth were observed on the inner side of the ocellus (pl. I, fig. 2). The teeth appear to be slightly below the level of the valve surface. The slits in the undescribed form from Kamischev showed similar sets of teeth (pl. II, fig. 8), and upon careful examination the same structure was revealed in *Fenestrella convexa* Brun (pl. I, fig. 4). Specimens of *Janischia antiqua* Grun. also show teeth on the inner side of the ocellus (pl. II, fig. 6), and careful examination showed that the lines of striæ form a bar across the valve similar to that in the other species being discussed. Inspection of Greville's original type specimen of *Fenestrella barbadensis* showed that it possessed similar teeth, and it was apparent that Greville had idealized his illustration. The specimen does not lie flat on the slide and is partly obscured, and the few apparent marginal puncta which could be seen seemed to be due to silhouettes of the terminal cellules of some of the radial striæ. Also, no trace was to be seen of the clear ring which Greville shows between the circle of puncta and the rim of the valve. Kamischev specimens of *F. barbadensis* Grev. show clearly a ring of what may be called sub-ocelli, from which rows of cellules seem to start (pl. I, fig. 1). These may also be seen in the type specimen, but are not illustrated by Greville. Similar sub-ocelli occur in *Janischia antiqua* Grun. (pl. II, fig. 5), *Fenestrella convexa* Brun. (pl. I, fig. 3) and the new species from Kamischev (pl. II, fig. 7). It is clear from all these points of resemblance that these species all belong to the same genus and that *Janischia antiqua* Grun. must accordingly be regarded as a synonym of *Fenestrella* Grev.

Regarding *Fenestrella gloriosa* Brun, there can be no doubt that this form is wrongly placed in this genus. Brun's original illustration shows all the lines of striæ proceeding in curved lines from the rim of the valve and merely forming an apparent bar between the ocelli. Schmidt (1894) figures forms which are similar to Brun's under the generic names *Cocconeis* (Janisch's identification) and *Rhaphoneis* (his own identification). One of the specimens is Brun's and from Oamaru, which suggests that a correction of classification is being attempted. An examination of a specimen mounted by Truan in a type slide of Oamaru diatoms in the British Museum collection indicates that there is no doubt that *Fenestrella gloriosa* Brun was wrongly named and should have been placed in the genus *Rhaphoneis*.

Recently a specimen of *Janischia antiqua* Grun. has been found in the Moreno shale of Panoche, California by Mr. Brigger and sent to Mr. Meakin. As this is the oldest deposit containing species of this group, Grunow's specific name is very appropriate, for it would certainly seem that this is the parent form of the genus. An intermediate form between *Fenestrella barbadensis* Grev. and *Fenestrella convexa* Brun has been reported in letters from New Zealand as occurring in the

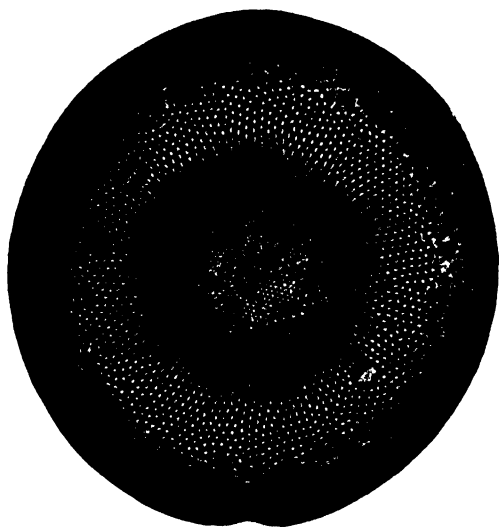


FIG. 1.



FIG. 2.



FIG. 3



FIG. 4.

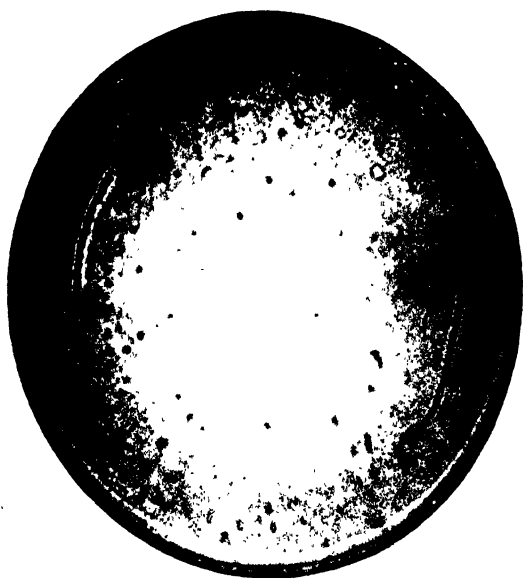


FIG. 5.

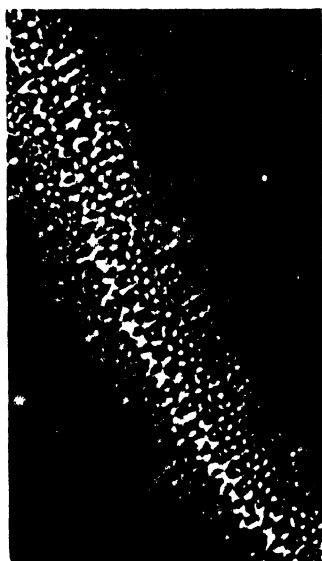


FIG. 6.

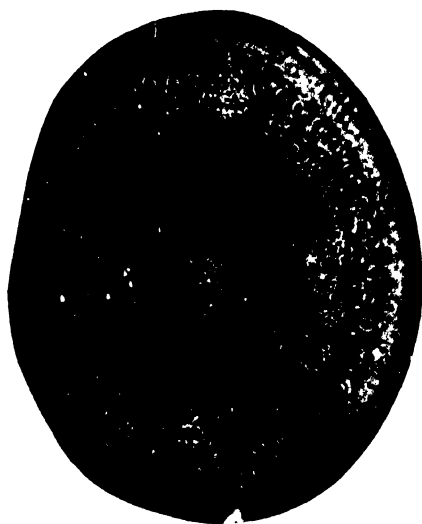


FIG. 7.



FIG. 8.

Oamaru deposits, but as no specimen is available it cannot be determined whether it should be classed as a separate species or as a variety of one of these forms.

An expanded description of the genus *Fenestrella* and a synopsis of the species contained therein, including a description of the new one from Kamischev, follows :—

Fenestrella Greville in *Trans. Microsc. Soc.*, London, n.s., **11**, 67 (1863).

Janischia Grunow apud Van Heurek, *Syn. Diat. Belg.*, pl. 95 bis, figs. 10, 11 (1882).

Valves circular or somewhat oval, convex, with radial punctuation. Two circular or linear ocelli are situated at approximately two-thirds of the radius from the centre on opposite sides of the valve. Lines of striæ proceed from these ocelli towards the central area, meeting in an irregular line at right-angles to the line joining the ocelli. At the inner side of the clear ocelli and slightly below the surface of the valve, lines of puncta, looking like sets of teeth, occur. A ring of sub-ocelli, from which lines of puncta start, is situated slightly further from the centre than the ocelli. These lines of puncta extend to the edge of the main bar of striæ and do not reach to the centre of the valve.

Fenestrella barbadensis Grenville, l.c., p. 68.

Fenestrella convexa Brun in *Mém. Soc. Phys. Hist. Nat. Genève*, **31**, **2**, **1**, 27 (1891).

Fenestrella antiqua (Grunow apud Van Heurek) Swatman comb. nov.

Janischia antiqua Grunow apud Van Heurek, l.c.

Coscinodiscus ludovicianus Rattray in *Proc. Roy. Soc.*, Edinburgh, **16**, 596 (1890).

Fenestrella russica Gwatman, spec. nov.

Valve large, convex, circular or somewhat oval (diam. 200–250 μ), finely punctate with the puncta disposed in quincunx, 9 in 10 μ at the centre of the valve, becoming closer towards the margin. Two ocelli are situated at approximately two-thirds of the radius from the centre, subtending an angle of approximately 10°. A row of puncta or “teeth” can be seen projecting into the inner edge of these clear ocelli. A ring of sub-ocelli occur at a slightly greater distance from the centre and from these, as from the main ocelli, fascicles of striæ appear to start. Only the fascicles from the main ocelli, however, reach the centre, where their junction forms a more or less well-defined line at right-angles to the line joining the ocelli.

Type in Herb. Mus. Brit., coll. Diat. no. B.M. 36048.

Species excluded :—

Fenestrella gloriosa Brun, l.c.

I wish to express my thanks to Mr. Meakin of Sheffield, who provided some of the mounted specimens examined in this work, to Mr. R. Ross of the Botanical Department, British Museum (Natural History) for facilities to examine Greville's type specimen, and to Mr. N. Ingram Hendey, Admiralty Central Metallurgical Laboratory, who prepared the illustrations.

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DESCRIPTION OF PLATES.

PLATE I.

- Fig. 1.—*Fenestrella barbadensis* Grev., Kamischev, Russia. × 675.
- Fig. 2.—*Fenestrella barbadensis* Ocellus showing "teeth." × 5000.
- Fig. 3.—*Fenestrella convexa* Brun, Springfield, Barbados. × 675.
- Fig. 4.—*Fenestrella convexa* Ocellus showing "teeth." × 5000.

PLATE II.

- Fig. 5.—*Fenestrella antiqua* (Grun. apud Van Heurck) Swatman, Jutland. × 275.
- Fig. 6.—*Fenestrella antiqua* Ocellus showing "teeth." × 2000.
- Fig. 7.—*Fenestrella russica* Swatman, Kamischev, Russia. × 325.
- Fig. 8.—*Fenestrella* showing ocellus and central area. × 700.

ABSTRACTS

CYTOLOGY.

Tests for Plant Virus Diseases.—R. C. LINDNER ("A Rapid Chemical Test for Some Plant Virus Diseases," *Science*, 1948, **107**, 17–18). A reagent composed of 40 g. sodium hydroxide, 0.3 g. cupric sulphate, 3.0 g. sodium citrate and 1000 ml. water is found to give definite colour reactions with many of the virus diseases of cherry and peach trees. Leaf tissue in the form of discs 6 mm. diameter is treated on a water bath with 5 ml. of the reagent for 5–10 minutes, allowed to cool 10 minutes, and studied in a colorimeter. Normal leaf tissue yields a blue-green to yellow-green colour; certain viruses give varying intensities of red. Maximum absorption differences between normal and virus-diseased tissues occur in the region 450–525 m μ . The mechanism of the test is probably due to polyhydroxy phenols of the tannin group which are formed by the virus in the phloem of the plant.

A. E. J. V. & K. V.

Fungoid Diseases of Man.—M. GUMPERT ("Histoplasmosis," *Scientific American*, 1948, **178** (6), 12–15, 4 figs.). Histoplasmosis is a fungus disease which has many of the attributes of tuberculosis. It is caused by the fungus *Histoplasma capsulatum*. Little is known about systematic fungus infections. Histoplasmosis causes lung calcifications. It would appear that tuberculosis has often been diagnosed when histoplasmosis would have been correct. The causal fungus appears in two varieties, one harmless, the other parasitic and the causal organism of histoplasmosis. The method of spread is unknown. Droplet infection is considered likely. The fungus shows an affinity for the cells of reticulo-endothelial system. Christie's skin test uses an antigen from a culture of *H. capsulatum*. Emmons has questioned the test, as he found it positive in coccidioidomycosis and other fungus diseases. The use of penicillin and streptomycin for attacking bacteria may stimulate this threat from a new group of disease-causing organisms.

A. E. J. V. & K. V.

Vasomotor Activity Study.—C. A. RICHINS ("Use of the Freezing-Drying Technique for the Study of Vasomotor Activity," *Science*, 1948, **107**, 25). Direct microscopical examination of the smallest arteries and arterioles responding to stimulation is found possible by using freezing-drying following intravenous administration of autonomic drugs with vasomotor responses. For example, freezing the duodenal loop of rats in a Dewar flask with an acetone-solid carbon dioxide mixture following drug administration is found to prevent the vasomotor picture. The method is suggested for use in studies of vasomotor activity and similar histophysiological phenomena.

A. E. J. V. & K. V.

Fowl Leucosis.—E. P. JOHNSON ("Nitrogen Mustards in Fowl Leucosis," *Science*, 1948, **107**, 40–42, 1 fig., 10 refs.). Methyl-bis(chloroethyl)amine (NH₂) and tris(chloroethyl)amine (NH₃) injected intravenously have a profound action upon the immature

cells spoken of as hæmocyto blasts in hæmocyto blastic leucosis of fowls. A retardation of mitotic activity both in the marrow and peripheral blood was demonstrated 24 hours after treatment. There was some evidence that the mustards have a lethal effect upon the virus causing the disease in addition to causing the death of the proliferating cells.

A. E. J. V. & K. V.

Molecular Constitution and Pathogenicity.—WILLIAM SEIFRIZ ("Pathogenicity and Isosterism," *Science*, 1948, **107**, 15–16). There is a connection between toxicity and the isosteric properties of substances. In considering toxicity, similarity of physical relationships such as isomorphism, electronic arrangements, surface tension, viscosity, spectra appear to be more important than similarity of molecular structure. Experiments have been carried out with simple material—*Physarum polycephalum*; the results may be difficult to apply to complicated living organisms.

A. E. J. V. & K. V.

HISTOLOGICAL AND CYTOLOGICAL TECHNIQUE.

Improved Aceto-Carmine Staining.—G. BERTANI ("Manipulation of Tissues for Improved Aceto-carmine Staining," *Stain Technol.*, 1948, **23**, 117–18). To obtain satisfactory aceto-carmine smears the structural make-up of the tissues must be studied. The salivary glands of the larvæ of a species of *Phoridae* give poor staining owing to the central bulk of secretion. By breaking the glands with needles and by extracting the bulk of secretion during the glands' stay in the aceto-carmine before crushing, much better results are obtained.

G. M. F.

Selective Staining of Avian Embryoes.—R. M. TRUE ("Elimination of Fat and Protein prior to the Selective Staining of Bone," *Stain Technol.*, 1948, **23**, 119–22 3 refs.). The skeleton of the foetal bird is often difficult to stain because of the large amount of fat and protein. The method is specially recommended for 21-day-old chicks. The specimen either fresh or fixed (not in formalin, chromic acid or salts of heavy metals) is placed in equal parts of glycerol, 95 p.c. alcohol, and distilled water. Filtered pepsin solution is injected into the yolk-sac and incubated at 40° C. for 2–3 hours. The pepsin solution is made by dissolving 10 g. of pepsin in 100 ml. of distilled water and one drop of 6 N HCl. The specimen is then transferred to 5 p.c. aqueous potassium hydroxide solution to which has been added a few drops of 2 p.c. hydrogen peroxide. The mapped fat areas are injected with cellosolve. The embryo is left in the solution till the skeletal elements are clearly visible through the surrounding tissues. It is then transferred to dilute (0.0025–0.01 p.c.) alizarin red S in 2 p.c. aqueous potassium hydroxide till the desired degree of staining is obtained. If necessary the mapped fat areas can be reinjected with cellosolve. The specimen is then passed through glycerol (72 hours); 25 parts glycerol, 75 parts 2 p.c. aqueous potassium hydroxide: 50 parts glycerol, 50 parts 2 p.c. aqueous potassium hydroxide: 75 parts glycerol, 25 parts 2 p.c. aqueous potassium hydroxide. The embryo is then stored in glycerol to which a crystal of thymol has been added. Bone stains red: muscle is transparent and unstained: fat deposits are eliminated and the yolk-sac cleared.

G. M. F.

Clearing Brain Tissue.—J. HABERMAN ("Clearing Brain Tissue with Anethole," *Stain Technol.*, 1948, **23**, 143–4, 5 refs.). Anethole (C₁₀H₁₂O) is used for clearing the brain. The tissue is immersed in 100 p.c. alcohol for 1–2 days and kept at 60° C. by placing in an incubator. Frequent changes of solution are advisable and the remaining

lipid removed by ether. A piece of tissue 1 cm. in thickness will be extracted if left in ether for 2 days after the alcohols. Anethole has a refractive index of 1.5614, is colourless, slightly soluble in water, soluble in alcohol and ether, and liquid at ordinary temperatures. Larger pieces of tissue such as $4 \times 6 \times 3$ cm. must be kept in warm 100 p.c. alcohol for a week and in ether for 1-2 weeks. G. M. F.

Maceration of Plant Tissues.—L. A. HOHL ("Application of Pectic Enzymes to Maceration of Plant Tissues for Microscopic Study," *Stain Technol.*, 1948, **23**, 129-32, 6 refs.). A commercial preparation pectinol W as well as pectic enzymes secreted by growing *Aspergillus awamori* or *A. carbonarius* upon pectin-containing media were effective in macerating a variety of plant materials such as petioles, fruits, and fruit skins. G. M. F.

Staining Radulae.—B. VERDCOURT ("The Staining of Radulae," *Stain Technol.*, 1948, **23**, 145-9, 7 refs.). The various methods of staining and mounting radulae are reviewed. Radulae should be extracted with 0.5 to 1 p.c. sodium hydroxide solution and the associated tissues removed before staining. Two staining methods are recommended. Newly formed teeth and the bases of older ones are well stained by saturated aqueous chlorazol black E for up to 10 minutes. A more uniformly stained, preparation in which the cusps of all but the young teeth are alone stained, may be obtained by using the "oxidation-dahlia technique." The radula is first oxidized in N/10 potassium permanganate solution until black, and then decolourised in saturated aqueous oxalic acid. It is then stained in 0.1 p.c. aqueous solution of dahlia (Hofmann's violet), the staining time varying from 10 to 30 minutes, according to the material. The radula is then dehydrated and passed through xylol and clove oil into Canada balsam. Other mounting media can be used, but glycerin jelly is suitable only for the rapid examination of unstained radulae. Several other staining methods are briefly discussed and precautions to be observed while mounting are detailed. G. M. F.

A Tri-basic Stain.—R. SPOERRI ("A Tri-basic-dye Stain for Nerve Cells," *Stain Technol.*, 1948, **23**, 133-5, 4 refs.). This technique entails the use of a tri-basic stain which is prepared as follows: Cresylecht violet 2 g., toluidine blue 1 g., thionin 0.5 g., and 30 p.c. ethyl alcohol 200 ml. Tissues may be fixed in 10 p.c. formalin, 95 p.c. alcohol, Bouin, or 10 p.c. formalin followed by Bouin. Paraffin sections cut preferably at 4μ are mounted on slides by the starch medium and passed through xylol, absolute alcohol, 95 p.c. and 80 p.c. alcohol. Slides are dipped into the staining solution heated to 80-90° C. for 5-10 seconds. The primary differentiation is carried out for 1 second in acidified distilled water (10 drops of concentrated H_2SO_4 or HNO_3 in 200 ml. of distilled water); slides are dipped in distilled water for 1 second; differentiated in 80 and 90 p.c. alcohol for 1-2 seconds each; returned to 80 per cent. alcohol for 1 second; dipped and agitated in the still warm tri-basic stain solution for 1-2 seconds; rinsed in distilled water, dehydrated in 80, 95 and 100 p.c. alcohol, xylol, and mounted. The stain may be repeated returning to 80 p.c. alcohol before immersion in the dye solution. The cell bodies stain dark purple, axons, dendrites and nerve endings blue to reddish. Granules are purple or reddish. Nuclei and nucleoli are well differentiated. G. M. F.

A New Method of Decalcification.—H. K. KRISTENSEN ("An Improved Method of Decalcification," *Stain Technol.*, 1948, **23**, 151-4, 5 refs.). Formic acid is used for decalcification in the form of equal parts of 8N formic acid and 1N sodium formate

(pH 2.2). This mixture produces decalcification in just under 24 hours. Tissues are rinsed in running water for 24 hours and dehydrated in ascending strengths of alcohol, cedar oil, and ligroin. Tissues are embedded in paraffin. This method involves complete decalcification by a mixture with as high a pH as possible without decreasing the stainability of the Nissl granules by Einarson's progressive staining method, using gallocyanin (L. Einarson, 1935, *J. comp. Neurol.*, **61**, 101). G. M. F.

Triphenyltetrazolium Chloride.—R. PRATT and J. DUFRENOY ("Triphenyltetrazolium Chloride, a Valuable Reagent in Stain Technology," *Stain Technol.*, 1948, **23**, 137–41, 19 refs.). A 0.5 p.c. aqueous solution of 2, 3, 5 triphenyltetrazolium chloride is used to detect histochemically in plant tissues the sites of dehydrogenase activity, thus enabling a correlation of the dehydrogenase activity with the apparent redox potential of the triphenyltetrazolium chloride $\rightleftharpoons$ formazan equilibrium which is poised about -0.08 volts. This system operates in a physiological range since glucose dehydrogenase-coenzyme I, in the presence of its substrate, reduces the tetrazolium salts at pH 6.6. In penicillin assay plates flood with an 0.5 p.c. solution of 2, 3, 5 triphenyltetrazolium chloride; the zones of inhibition are outlined by a bright red ring. G. M. F.

An Elliptometer.—J. D. HAMILTON and M. L. BARR ("An Elliptometer: A Simple Method of Measuring the Areas of Structures in Microscopic Sections," *Stain Technol.*, 1948, **23**, 123–7, 4 figs.). A simple method of measuring the cross-sectional area of objects in microscopic sections is described and figured. The elliptometer consists of a source of light such as a good microscope lamp, an adjustable camera diaphragm, a lens, and a paper screen ruled in millimetre squares and adjustable around a horizontal axis. The lens must be of short focal length. Camera lucida tracings of the cells to be measured are used. The tracing is laid on the screen and the diaphragm and screen angle adjusted till the best "fit" is obtained. On removal of the tracing the lengths of the major and minor axes are read directly from the ruled paper. From these data figures for the mean major and minor axes of the cell group studied may be obtained. The mean cell cross-sectional area is determined from the formula for the area of an ellipse, πab , where a is the major semi-axis and b the minor semi-axis. The same data yield information regarding the mean ellipticity of the cells (b/a). G. M. F.

Spore-contrast Staining with Oxypyrene Trisulphonate.—J. MAY ("Einfache Sporenkontrastfärbung mit Oxypyrenetrisulfosäure," *Zbl. Bakt. I. Abt. Orig.*, 1947, **152**, 56–7). The sodium salt of 3-oxypyrene-5, 8, 10-sulphonate is employed to demonstrate spores and bipolar bodies. The alkaline solution of the dye produces a green, the acid solution a blue luminescence with intense prominence of spores and bipolar bodies when viewed in ultra-violet light. If spore-bearing bacteria are treated with mixtures of acid oxypyrene and acridine orange the bacteria shine reddish-brown and the spores blue in ultra-violet light. G. M. F.

Staining Heinz Bodies.—S. H. WEBSTER, E. J. LILJEGREN, and D. J. ZIMMER ("Rapid Staining of Heinz Bodies in Smears," *Stain Technol.*, 1948, **23**, 97–8, 1 fig., 6 refs.). Heinz in 1890 described colourless refractile globules of varying sizes in the red cells of animals caused by certain chemicals such as phenylhydrazine and its derivatives, aniline and sulphanilamide. Supravital methods have some advantages since an accurate count can be made, but the erythrocytes of some species are rapidly altered when subjected to this treatment. By using an 0.2 p.c. solution of methyl violet

(anilin violet, dahlia B; total dye content 88 p.c.) in 95 p.c. ethyl alcohol smears can be fixed and stained simultaneously. Crystal violet with a total dye content of 95 p.c. can also be used. A freshly prepared air-dried moderately thick blood-smear is covered with the dye solution and allowed to stain for one half-minute: the slide is then washed with running water. The Heinz bodies show up as blue granules, many of which are extracellular. G. M. F.

Uses of Periodic Acid.—J. F. A. McMANUS ("Histological and Histochemical uses of Periodic Acid," *Stain Technol.*, **23**, 99–108, 19 refs.). Carbohydrates and carbohydrate compounds can be demonstrated in tissue sections by periodic acid solutions. Periodic acid acts on 1, 2 glycols (R-CHOH-CHOH-R) to form aldehydes and these aldehydes are coloured by Schiff's reagent as in Feulgen's test. Ordinary Zenker-formol or Helly's fixative is satisfactory for fixing tissue and the usual dehydration routine of alcohol and toluene or xylene can be followed. For frozen sections the formol-saline technique of fixation can be used and a fat stain can be superimposed on a periodic acid preparation in frozen sections, using the usual Sudan IV or Sudan black techniques. The following technique for paraffin sections is provided: carry paraffin sections to water; wash in running tap water for 5 minutes if iodine and hypo were used; 0.5 p.c. periodic acid in water for 5 minutes; rinse in distilled water; Schiff's reagent for 15 minutes; rinse in three changes of sulphurous acid, each 2 minutes; wash in running water for 3–5 minutes; stain in Harris's hæmatoxylin for 20–30 seconds; wash in running water for 5 minutes; dehydrate in two changes of 95 p.c. alcohol; two changes of absolute alcohol; clear in xylene and mount in balsam. Schiff's reagent is prepared as follows: Boil 200 ml. of distilled water; add 1 gm. of basic fuchsin; cool to 50°C .; filter; add 20 ml. NHCl (98.3 ml. of HClSG 1-16 , made to 1000 ml. with distilled water); cool to 25°C . and add anhydrous solution bisulphite 1 gm. The fluid must be kept in the dark: after 2 days it becomes orange or straw-coloured and is then ready for use. The sulphurous acid rinse is made from 10 p.c. sodium metabisulphite, 6 ml.; normal hydrochloric acid 5 ml.; distilled water 100 ml.

For frozen sections: place in 0.5 p.c. aqueous periodic acid for 5 minutes; wash in several changes of distilled water; Schiff's reagent for 10 minutes; rinse in three changes of sulphurous acid, each for 2 minutes; tap water; counterstain, if desired with Harris' hæmatoxylin for 20–30 ml.; wash well in water; mount in glycerol or glycerol jelly.

Mucin, hyaluronic acid and mucin are coloured: hyaluronic acid is best preserved after overnight fixation in 4 p.c. lead subacetate. Basement membrane, reticulin, the colloid of the pituitary stalk and of the thyroid gland, some of the acidophilic cells of the human anterior hypophysis and the granular cells of the renal arteriole are coloured. In pathological material many "hyaline" materials are coloured—amyloid infiltrations, arteriosclerotic hyaline, colloid droplets and mitotic figures. The histochemical uses of the periodic acid-Schiff's reagent need careful control because of the possibility of attachment of iodate or periodate to tissue constituents, producing a recolouration of the Schiff's reagent. Whenever possible the positive reacting material should be identified by other methods. G. M. F.

Staining Plant Tissues.—R. M. REEVE ("An Adaptable Staining Schedule for Plant Tissues," *Stain Technol.*, **23**, 13–15, 8 refs.). A general schedule for staining meristematic, maturing, and mature plant tissues is described. Paraffin sections are treated with xylol, absolute alcohol, and 95 p.c. ethyl alcohol. They are then rinsed in water which may contain a mordant and placed for 5–15 minutes in Delafield's

hæmatoxylin diluted to about 10 p.c. Overstaining should be avoided. After rinsing in water sections are stained in safranin O, 0.1 p.c. in 60 p.c. ethyl alcohol with a few drops of 5 p.c. aqueous sodium acetate. From 30 minutes to 2 hours are required but staining may be hastened by the use of a warm solution in a paraffin oven for 5-15 minutes. Sections are rinsed in water and destained in alcohol. Fast green serves as a destaining agent as well as a counterstain. A few millilitres of a saturated solution of fast green FCF in clove oil and alcohol (1 : 1) are added to a Coplin jar containing 50 ml. xylene and alcohol in a ratio of 3 : 1; 1-3 minutes is usually long enough to complete the destaining of the safranin and to provide a good counterstain. Rinse in xylene-alcohol and in two changes of xylene before mounting in balsam.

G. M. F.

Paraffin Infiltration.—E. K. DOXTADER (“*iso*Prophyl Alcohol in the Paraffin infiltration technique,” *Stain Technol.*, 1948, **23**, 1-2, 4 refs.). Formalin-fixed animal tissues are washed in running water overnight to remove the fixative. They are dehydrated, infiltrated, and embedded as follows: *isoprophyll* alcohol 60 p.c., 1 hour; *isoprophyll* alcohol 99 p.c., 1 hour; *isoprophyll* alcohol 99 p.c., 2 hours; *isoprophyll* alcohol 99 p.c. (40-45° C.), 3 hours; melted tissuemat (melting-point 50-52° C.), 1 hour; melted tissuemat (melting-point 50-52° C.), overnight. Embed and block in tissuemat of the correct melting-point to suit cutting conditions. Sections can be cut 8 μ thick.

G. M. F.

Staining Bone.—C. O. BECKTOL (“Differential *in toto* Staining of Bone, Cartilage, and Soft Tissues,” *Stain Technol.*, 1948, **23**, 3-8, 12 refs.). To study foetal skeletal deformities it was advantageous first to study the specimens grossly with the bone and cartilage stained and the soft tissues transparent, and thereafter to follow this with microscopic examination of the same specimen. The liver must be first removed as it is impossible to bleach. The staining and destaining process depends on diffusion of solutions through the tissues: it is facilitated by complete evisceration and by opening the cranial cavity through the fontanelles. Fix well in neutral 10 p.c. formalin with calcium carbonate: bleach thoroughly in several changes of 3 p.c. hydrogen peroxide in distilled water in sunlight. The bleaching process may take several weeks. Wash for 24 hours in distilled water. Optimum dye concentrations must be used. For bone stains 0.1 gm. per litre are used of benzopurpurin, Biebrich scarlet, Bordeaux red, eosin 7, fast green FCF; Ponceau red; orange II is best with 0.5 gm. per litre. For cartilage stains citric acid is added to the extent of 21 gm. per litre and 0.02 gm. per litre of the following stains is employed, cresyl echt violet, gentian violet, Janus green, Nile blue, thionin, toluidin blue and victoria blue: 0.01 gm. of methylene is employed. For bone stains, stain for 24 hours; then place in alcohol 60-95 p.c. till the soft tissues and cartilage are colourless. For cartilage stains are used for 24 hours: destaining is carried out in pH 2 buffer till the soft tissues are pale. The final dehydration with 95 p.c. alcohol must not be too prolonged. The bone is first stained and destained and then the cartilage is stained and cleared. The tissues are dehydrated in two changes of 95 p.c. alcohol and then placed in Groat's cleaning fluid. Groat's mixture 19 is satisfactory. Cover loosely so that the alcohol may evaporate or the alcohol may be evacuated *in vacuo*. Groat (*Stain Technol.*, 1941, **16**, 111) achieves maximum clearing by adjusting the refractive index of a mixture of triorthocresyl phosphate and tributyl phosphate to that of the tissue. Tissues may be infiltrated directly from the triorthocresyl and tributyl phosphate mixture. Sections must be restained as the processing removes most, if not all, of the *in toto* stain.

G. M. F.

A Modification of the Kohler System of Illumination.—D. E. COPELAND (A Utilization of the Kohler System of Illumination, *Stain Technol.*, **23**, 9–11, 2 refs.). The method is an implementation of Kohler's technique. The light source is a 6-volt ribbon filament lamp in a housing equipped with condensing lenses and an adjustable diaphragm. If a silvered mirror or other reflector is located in the lamp, it must be removed so that the only effective illumination is directly from the ribbon filament. The diaphragm of the lamp is opened and the image of the incandescent ribbon filament focused on the diaphragm of the substage condenser. The diaphragm is then closed to about one half-inch diameter. The material on the slide is brought into focus. The substage condenser is adjusted until the edges of the lamp diaphragm are in focus. The lamp diaphragm is opened to just beyond the visual field. The condenser diaphragm is adjusted to give the best resolution, avoiding glare and diffraction bands. The polaroid elements are adjusted to obtain a satisfactory intensity of light. Filters are inserted for the desired contrast and the polaroid settings readjusted.

G. M. F.

Rotary Microtomes.—R. P. COWLES and O. W. RICHARDS ("The Pfeifer and Minot Automatic Rotary Microtomes," *Trans. Amer. micr. Soc.*, 1947, **66**, No. 4, 379–82, 7 refs.). The early history of rotary microtomes is discussed. C. S. Minot designed a microtome on rotary principles about 1886; this instrument with slight modifications is still in use to-day. Another automatic rotary microtome was designed probably about 1879 by Adam Pfeifer of the Biological Laboratory of the Johns Hopkins University. This microtome was never put on the market, but several examples still exist in America: their mechanism is described and figured.

G. M. F.

Methylene Blue and Eosin for Sections.—R. L. MAYHEW ("A Method for Using Methylene Blue and Eosin as a Stain for Sections," *Trans. Amer. micr. Soc.*, 1947, **66**, No. 4, 398–9). The main difficulty is to retain enough eosin during the differentiation process to give satisfactory coloration. Sections are brought down from xylol through alcohols to distilled water. They are then stained for 3–5 minutes in a solution containing 1 g. of methylene blue and 1 g. of borax in 100 ml. of distilled water. Wash off excess stain with distilled water and pass slides through 50 and 70 p.c. alcohol. Differentiate in 85 p.c. alcohol to which a few drops (4–5–50 ml.) of resin in absolute alcohol are added. Destaining is rapid. Wash the slides in 80 and 95 p.c. alcohol. Counterstain in eosin in 95 p.c. alcohol. The eosin is made by dissolving 0.5 g. in 100 ml. of 95 p.c. alcohol. For some tissues this solution can be diluted 1 in 2, but 1 in 4 or 1 in 5 dilution may be required. A few seconds is all that is necessary for staining. Zenker fixed tissue is satisfactory.

G. M. F.

PROCEEDINGS OF THE SOCIETY.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, OCTOBER 16TH, 1946, AT 5.30 P.M., DR. JAMES A. MURRAY, F.R.S., PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed and signed by the President.

New Fellow.—The following Candidate was balloted for and duly elected an Ordinary Fellow of the Society:—

A. H. Collinson.

Worthing.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner:—

G. D. Bhavnani.
F. L. Channing.
J. H. Fodden.
T. A. M. Gerrard.
E. J. Hanson.
A. C. Hurt.
J. Maingard.
G. Mallett.
E. A. Phoenix.
J. Potter.
P. K. Sanyal.
G. Smith.
B. Verdcourt.

London.
Feltham.
Shrewsbury.
New Barnet.
London.
Los Angeles.
Mauritius.
Bristol.
Loughborough.
Glasgow.
London.
Harrow.
Luton.

Deaths.—The Chairman announced the regrettable loss to the Society, by death, of the following Fellows:—

A. Barrett.
R. Hagelstein.
A. E. Merlin.
B. Stracey.

Elected 1923.
„ 1923.
„ 1899.
„ 1938.

Donations were reported from:—

Mr. Geoffrey Cumberlege—

“Principles of Micropalæontology,” by Martin F. Glaessner.

The University of North Carolina Press—

“Studies in Science,” edited by W. C. Coker.

M. Paul Lechevalier—

“Faune de France,” No. 43, Celeoptres Bruchides et Anthribides, by Adolphe Hoffmann.

“Faune de France,” No. 44, Insectes Extoparasites, by Eugene Seguy.

Executors of the late Mr. F. J. Carrel—

Nine pounds ten shillings.

Dr. Gunnar Svardson—

“Chromosome Studies on Salmonidæ,” by Dr. Gunnar Svardson.

Mrs. F. Coles—

“Microscope Technique,” a selection of letters between E. M. Nelson and A. C. Cole, edited by F. W. Watson Baker.

Mr. C. L. Curties, F.R.M.S.—

Dollond Microscope.

Mr. J. H. Wredde, F.R.M.S.—

“Varnished Cloths for Electrical Insulation,” by H. W. Chatfield and J. H. Wredde.

Messrs. Food Trade Press Ltd.—

“The Microscope and its Use,” by F. J. Munoz in collaboration with H. A. Charriper.

Paper.—The following communication was read and discussed:—

Mr. E. Wilfred Taylor, C.B.E., F.Inst.P., F.R.M.S.—

“Improved Image Illumination and Contrast with the Metallurgical Microscope.”

A vote of thanks was accorded to Mr. Taylor for his communication.

Announcement.—The Secretary made the following announcement:—

The next meeting of the Biological Section will be held on Wednesday, November 6th, 1945.

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, NOVEMBER 20TH, 1946, AT 5.30 P.M., DR. JAMES A. MURRAY, F.R.S., PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed and signed by the President.

New Fellows.—The following candidates were balloted for and duly elected Ordinary Fellows of the Society:—

G. D. Bhavnani.	London.
F. L. Channing.	Feltham.
J. H. Fodden.	Shrewsbury.
T. A. M. Gerrard.	New Barnet.
E. J. Hanson.	London.
A. C. Hurt.	Los Angeles.
J. Maingard.	Mauritius.
G. Mallett.	Bristol.
E. A. Phoenix.	Loughborough.
J. Potter.	Glasgow.
P. K. Sanyal.	London.
G. Smith.	Harrow.
B. Verdcourt.	Luton.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner:—

W. H. J. Coombs.	Sheffield.
E. F. Daglish.	Aylesbury.
P. Goodell.	Springfield, U.S.A.
L. G. S. Mason.	Orpington.
L. Bilton.	Rotherham.

Death.—The President announced the regrettable loss to the Society, by death, of the following Fellow:—

A. Reid.	Elected 1910.
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Paper.—The following communication was read and discussed:—

Mr. F. C. Grigg, F.L.S., F.R.M.S.—

“Chromosome and Cellular Mechanism and the Analysis of the Cell as a Structural Unit.”

A vote of thanks was accorded to Mr. Grigg for his communication.

Announcement.—The Secretary made the following announcement:—

The next meeting of the Biological section will be held on Wednesday, December 4th, 1946.

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, DECEMBER 18TH, 1946, AT 5.30 P.M., DR. JAMES A. MURRAY, F.R.S., PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed and signed by the President.

New Fellows.—The following Candidates were balloted for and duly elected Ordinary Fellows of the Society :—

L. Bilton.	Rotherham.
W. H. J. Coombs.	Sheffield.
E. F. Daglish.	Aylesbury.
P. Goodell	Springfield, U.S.A.
L. G. S. Mason.	Orpington.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner :—

S. Johnson.	Twyford.
W. H. Lawson.	Headingley.
P. D. C. Mumby.	London.
A. Stock.	London.

Death.—The President announced the regrettable loss to the Society by death, of the following Fellow :—

F. J. Tanner.	Elected 1932.
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Paper.—The following communication was read and discussed :—

Dr. H. V. Walters, A.R.C.S., D.I.C., B.Sc.

“ The Design and Use of Optical Colour Filters.”

A vote of thanks was accorded Dr. Walters for his communication.

Announcement.—The Secretary made the following announcement :—

The next Meeting of the Biological Section will be held on Wednesday, January 1st, 1947.

The Proceedings then terminated.

THE ANNUAL GENERAL MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, 15TH JANUARY, 1947, AT 5.30 P.M., DR. G. M. FINDLAY, C.B.E., D.Sc., VICE-PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed and signed by the Chairman.

New Fellows.—The following Candidates were balloted for and duly elected Ordinary Fellows of the Society :—

S. Johnson.	Twyford.
W. H. Lawson.	Headingley.
P. D. C. Mumby.	London.
A. Stock.	London.

Nomination Certificates in favour of the following Candidates were read for the first time and directed to be suspended in the rooms of the Society in the usual manner :—

R. K. Fleming.	Bedford.
A. R. N. Iyer.	Manchester.
A. W. Pettit.	Brentwood.
H. W. Pickworth.	Thornton Heath.

Deaths.—The Chairman announced the regrettable loss to the Society, by death, of the following Fellows :—

J. B. Capell.	Elected 1913.
H. Price.	Elected 1933.

ONE HUNDRED AND SEVENTH ANNUAL REPORT. REPORT OF THE COUNCIL FOR THE YEAR 1946.

The Report of the Council for the year 1946 was read by the Secretary :—

The past year marked the renewal of the full activities of the Society after the restrictions imposed by the Second World War. The Council can now report that, in spite of the difficulties resulting from the general world shortage of supplies and labour, a successful programme has been planned and carried out in all sections of the Society's activities.

FELLOWS.

During 1946 the Council has reported, with deep regret, the death of the following Fellows :—

A. Barrett.	Elected 1923.
J. A. Coultas.	„ 1927.
R. Hagelstein.	„ 1923.
A. E. Merlin.	„ 1899.
A. Reid.	„ 1910.
D. G. Rowson.	„ 1941.
B. Stracey.	„ 1938.
F. J. Tanner.	„ 1932.

Ten Fellows have resigned and ten have been removed from the Roll of Fellowship under By-law 31.

Two reinstatements have taken place and thirty-four new Ordinary Fellows, and one Honorary Fellow have been elected.

MEETINGS.

Nine Meetings of the Council and eight Ordinary Meetings, all of which have been well attended, have been held in the rooms of the Society.

JOURNAL.

Publication of the Journal has been continued and with the return of the Honorary Editor, Dr. G. M. Findlay, *C.B.E.*, it is anticipated that the work of bringing the Journal up-to-date will be completed within the next eighteen months. Increased costs of printing and shortages of paper supplies have made it necessary to continue the practice of issuing two parts under one cover. Whilst existing conditions prevail it is not anticipated that a reversion will be made to the usual practice of issuing the Journal quarterly, but this will, naturally, be done as soon as it is in any way practicable.

LIBRARY.

Thirteen new volumes on recent developments in microscopy and current researches have been added to the Library during the past year, in addition to the normal accessions received in exchange for the Society's publications. The thanks of the Fellows have been conveyed to the following donors :—

Messrs. Baillière, Tindall & Cox, Mrs. F. Cole, Mr. Geoffrey Cumberlege, Messrs. Food Trade Press, Ltd., M. Paul Lechevalier, Messrs. Longmans', Green & Co., Ltd., Dr. Gunnar Svardson, University of North Carolina Press, Dr. Paul Vonwiller, Dr. T. E. Wallis, *I.S.O.*, *F.R.M.S.*, Messrs. John Wiley & Sons, Inc., and Mr. J. H. Wredde, *F.R.M.S.*

The Library continues to be used by many Fellows and the National Central Library has also obtained a number of volumes therefrom.

INSTRUMENTS AND APPARATUS.

The following instruments have been added to the Society's historical collection and the thanks of the Fellows have been duly conveyed to the donors of these valued accessions :—

Mr. C. L. Curties, *F.R.M.S.*—

A "Dollond Microscope" with accessories in case.

Major Maxwell Knight, *F.Z.S.*, *F.R.C.S.*, *F.R.M.S.*—

An "Improved Compound Microscope" by Carpenter.

SLIDE CABINET.

Continued use has been made of the Society's collection of slides and the Curator reports that the reorganization of the cabinet continues.

Mr. F. C. Grigg, *F.L.S.*, *F.R.M.S.*, has presented thirty-six slides showing plant cell development and structure.

APPENDIX.

BIOLOGICAL SECTION.

The seven Ordinary Meetings of the Biological Section during the year were held in the Hastings Hall. A wide field was covered by the Communications and these, together with the miscellaneous exhibits, caused much interest and lively discussion.

New Council.—The Chairman appointed Mr. F. Welch and Mr. J. H. Wredde to act as Scrutineers of the ballot for the election of Officers and Members of Council for 1947; subsequently, upon receipt of the Scrutineers' report, the result of the Ballot was declared from the Chair as follows :—

President.—R. J. Ludford, *Ph.D.*, *D.Sc.*

Vice-Presidents.—G. M. Findlay, *C.B.E.*, *M.D.*, *D.Sc.*, C. F. Hill, *M.Inst.M.M.*, *A.Inst.P.*, *F.L.S.*, J. A. Murray, *M.D.*, *F.R.S.*, T. E. Wallis, *D.Sc.*, *F.I.C.*, *Ph.C.*

Hon. Treasurer.—S. R. Wycherley.

Hon. Secretaries.—F. C. Grigg, F.L.S., H. Moore, D.Sc., A.R.C.S., F.Inst.P.

Ordinary Members of Council.—F. D. Armitage, F.R.P.S., F.L.S., L. P. Clarke, T.D., M.R.C.S., D.P.H., F. Greenshields, B.Sc., Ph.D., Maxwell Knight, O.B.E., F.R.E.S., F.Z.S., F.R.G.S., Chetwynd Palmer, R. Ross, M.A., D. J. Scourfield, I.S.O., J. Smiles, A.R.C.S., H. Gilbertson Smith, C.B., O.B.E., M.C., E. Wilfred Taylor, C.B.E., F.Inst.P., C. Tierney, D.Sc., F.L.S., J. M. Watson, A.R.C.S., D.Sc.

Hon. Editor.—G. M. Findlay, C.B.E., M.D., D.Sc.

Hon. Librarian.—R. Ross, M.A.

Hon. Curator of Instruments.—C. Tierney, D.Sc., F.L.S.

Hon. Curator of Slides.—N. Ingram Hendey, M.P.S., F.L.S.

On the motion of the Chairman, a hearty vote of thanks was accorded to the Scrutineers for their services.

Donations were reported from :—

Exors. of the late J. Rheinberg—

Rheinberg's original differential colour-screen sub-stage devices—3.
A 14,000 lines per inch grating replica ridged surface.

Exors. of the late H. Taverner—

Taverner's original decentering stereo-photomicrographic nosepiece.

Mr. W. Williamson, F.R.S.E., F.R.M.S.—

A small 6-volt micro-slide projector.
A collection of plain and coloured lantern slides of mites.

Brigadier H. Gilbertson Smith, C.B., O.B.E., M.C., T.D.—

An old stage prism illuminator.
A sub-stage cylindrical lense with stop.
An old rack-focussing body tube (Culpepper Type) with four objectives.

Mr. F. C. Grigg, F.L.S., F.R.M.S.—

Smith & Beck Microscope with accessories—c. 1850.

A vote of thanks was accorded to the donors.

Paper.—In the regrettable absence of Dr. James A. Murray, F.R.S., President, who was unable to attend the Meeting owing to ill-health the following communication was read and discussed :—

Dr. R. J. Ludford, Ph.D.—

“Tissue interaction in tissue cultures.”

A vote of thanks was accorded to the author of the foregoing communication.

Announcement.—The Secretary made the following announcement :—

The Biological Section will meet in the Hastings Hall on Wednesday, February 5th, 1947.

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, FEBRUARY 19TH, 1947, AT 5.30 P.M., DR. R. J. LUDFORD, PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed and signed by the President.

New Fellows.—The following Candidates were balloted for and duly elected Ordinary Fellows of the Society :—

H. K. Fleming.	Bedford.
A. R. N. Iyer.	Manchester.
A. W. Pettit.	Brentwood.
H. W. Pickworth.	Thornton Heath.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner :—

R. J. Barnes.	Malaya.
A. Bloomer.	Atherstone.
W. W. Mackinlay.	Kippen.
A. G. Willis.	Manchester.
R. F. A. Fattah.	Manchester.

Donation was reported from :—

Mrs. L. E. Bowell—

Large Ross Binocular Stand with accessories in Mahogany Case.

Paper.—The following communication was read and discussed :—

Mr. E. Wilfred Taylor, *C.B.E.*, *F.Inst.P.*, *F.R.M.S.*—

“ The Practical Application of Phase-Contrast to the Biologist's Microscope.” followed by—

Dr. A. F. W. Hughes', *M.A.*—

“ Phase-Contrasts Films of Tissue Cultures.”

Thereafter a general discussion was opened by a short summary by Professor L. C. Martin, *D.Sc.*, followed by many questions from the audience.

A vote of thanks was accorded to Mr. Taylor, Dr. Hughes, Professor Martin and Miss Anthony of the Wellcome Foundation, who had kindly arranged the cinematograph projector.

Announcements.—The President made the following announcements :—

The next Meeting of the Biological Section will be held on Wednesday, March 5th, 1947.

Details of Meetings on the Electron Microscope on March 19th, 20th and 21st, 1947.

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, MARCH 19TH, 1947, AT 5.30 P.M., DR. R. J. LUDFORD, PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed and signed by the President.

New Fellows.—The following Candidates were balloted for and duly elected Ordinary Fellows of the Society :—

R. J. Barnes.	Malaya.
A. Bloomer.	Atherstone.
R. F. A. Fattah.	Manchester.
W. W. Mackinlay.	Kippen.
A. G. Willis.	Manchester.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner :—

F. M. Anthony.	London.
H. Dixon.	Chichester.
A. F. W. Hughes.	Cambridge.
K. Rosenbaum.	Greenford.
F. A. Silver.	Oakland.
V. H. Simonian.	London.
J. Smith.	S. Pasadena.
P. L. Stiles.	London.
R. H. S. Fox.	Woodford Green.
W. H. Thomas.	Abergavenny.

Paper.—The following communication was read and discussed :—

Mr. M. E. Haine, B.Sc., A.M.I.E.E.—

“The Design and Principles of the Electron Microscope.”

A vote of thanks was accorded to the author of the foregoing communication.

Announcements.—The President then made the following announcements :—

A two-day conference will be held with the Electron Microscope Group of the Institute of Physics on Thursday and Friday, March 20th and 21st, 1947.

The Biological Section will meet on Wednesday, April 2nd, 1947.

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, APRIL 16TH, 1947, AT 5.30 P.M., DR. R. J. LUDFORD, PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed and signed by the President.

New Fellows.—The following Candidates were balloted for and duly elected Ordinary Fellows of the Society :—

F. M. Anthony.	London.
H. Dixon.	Chichester.
R. H. S. Fox.	Woodford Green.
A. F. W. Hughes.	Cambridge.
K. Rosenbaum.	Greenford.
F. A. Silver.	Oakland (California).
V. H. Simonian.	London.
J. Smith.	S. Pasadena (California).
P. L. Stiles.	London.
W. H. Thomas.	Abergavenny.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner :—

T. Bennett.	London.
A. H. Edwards.	Chichester.
A. G. Sabin.	New Barnet.
M. E. Folan.	Cork.

Balance Sheet.—The President called upon the Treasurer to submit the Audited Accounts and Financial Statement for the year 1946.*

* Treasurer's Report and Financial Statement for the year ending December 31st, 1946, will appear in the next issue of the Journal.

BALANCE SHEET AS

	LIABILITIES.	£	s.	d.	£	s.	d.	£	s.	d.
<i>Capital—</i>										
Being (a) Life Compounded Subscriptions from 1st January, 1877 to 31st December, 1945		2336	13	0						
Add: Amount received during 1946		55	2	6						
					2391	15	6			
(b) Quekett Memorial Fund					100	0	0			
(c) Mortimer Bequest					45	0	0			
(d) A. N. Disney Bequest					100	0	0			
(e) Amount received in respect of Sales of Books and Catalogues from the Library (surplus to the Society's requirements) to 31st December, 1945		339	3	9						
Add: Sales during 1946		4	15	3						
					343	19	0			
(f) Admission Fees to 31st Decem- ber, 1945		688	16	0						
Add: Fees during 1946		65	2	0						
					753	18	0			
(g) A. Gandolfi Hornyold Bequest					100	0	0			
(h) F. Balfour-Browne—Gift					100	0	0			
					3934	12	6			
Less: Amount transferred to Super- annuation Reserve in respect of year ended 31st December, 1935					100	0	0			
								3834	12	6
<i>Sundry Creditors, Specific Reserves and Accrued Charges—</i>										
Subscriptions paid in advance					47	3	4			
Journal Subscriptions paid in advance					30	13	0			
Accrued Charges					3	10	4			
Reserve for Expenses in connection with the Centenary Meeting					211	14	0			
Reserve for Repairs to Instruments					100	0	0			
Reserve for cost of printing Journal, Illustrating, Postages and Reprints					1250	0	0			
								1643	0	8
Forward								£5477	13	2

AT 31ST DECEMBER, 1946.

	ASSETS.	£	s.	d.	£	s.	d.
<i>Furniture and Equipment—</i>							
As at 31st December, 1945	.	76	6	8			
Less : Depreciation at 10% per annum	.	7	16	8			
					70	10	0
<i>Investments at Cost, less amount written off—</i>					4415	10	3
£400 London and North Eastern Railway Co. 3% Debenture Stock.							
£500 Nottingham Corporation 3% Irredeemable Debenture Stock.							
£150 Metropolitan Water Board "B" Stock.							
£612 London Midland and Scottish Railway Co. 4% Preference Stock.							
£200 New South Wales 5½% Loan, 1947-57.							
£100 3% Conversion Loan, 1948-53.							
£421 1s. 3½% War Stock (Registered).							
£400 3½% War Stock (Inscribed).							
£300 Birmingham Corporation 3¼% Redeemable Stock, 1957-62.							
£400 3% Defence Bonds.							
£250 3% Savings Bonds.							
£600 3% Defence Bonds.							
£1200 3% Savings Bonds.							
<i>Note.</i> —The market value of the above investments at 31st December, 1946, was approximately £5718. In accordance with the Resolution of the Council dated 21st October, 1942, £900 3% Defence Bonds was earmarked in respect of the Superannuation Reserve.							
<i>Sundry Debtors—</i>							
Subscriptions and Journal Sales	.	590	3	3			
Less : Reserve for Doubtful Debts	.	405	6	0			
					184	17	3
<i>Cash at Bank and in Hand—</i>							
National Provincial Bank, Limited—							
Deposit Account	.	500	0	0			
Current Account	.	739	17	0			
In Hand	.	11	7	2			
					1251	4	2
Forward	.				£5922	1	8

BALANCE SHEET AS

LIABILITIES—*continued.*

	£	s.	d.	£	s.	d.
Forward	.	.	.	5477	13	2

Superannuation Reserve—

Annual Instalments and Interest as at 31st December,						
1945	1314	3	11			
Less : Cost of Annuity purchased	1314	0	0			
Transferred to Income and Expenditure						
Account		3	11			

Rehabilitation Reserve—

Amount transferred from Income and Expenditure				500	0	0
Account, subject to approval of Council						
				<u>£5977</u>	<u>13</u>	<u>2</u>

S. R. WYCHERLEY, *Hon. Treasurer.*

 AT 31st DECEMBER, 1946.

ASSETS—continued.

	£	s.	d.	£	s.	d.
Forward	.	.	.	5922	1	8

Income and Expenditure Account—

Excess of Expenditure over Income for the year ended
31st December, 1946

112 14 9

Less : Balance credit at 31st December, 1945

57 3 3

55 11 6

£5977 13 2

We have examined the Accounts and Balance Sheet with the Books and Vouchers of the Society, and having received all the information and explanations we have required, report that the above Balance Sheet is properly drawn up so as to exhibit a true and correct view of the Society's affairs at the 31st December, 1946, according to the best of our information, and the explanations given to us, and as shown by the books. We have verified the investments and Cash Balances.

(Signed) HUNTER, JONES, HALFORD & CO.,

Hon. Auditors.

45, Ludgate Hill, London, E.C.4.
27th March, 1947.

Dr.		INCOME AND EXPENDITURE ACCOUNT FOR							
1945.		EXPENDITURE.		£	s.	d.	£	s.	d.
£									
167	To Rent, Telephone and Insurance	.	.	.	.	.	168	9	10
350	„ Salaries	.	.	.	.	.	443	13	4
	„ Journal Expenditure—								
400	Printing—provision	.	.	.	.	.	290	0	0
	Printing—actual	.	.	.	.	.	220	12	8
159	Editing and Abstracting	.	.	.	.	.	53	0	0
35	Illustrating	.	.	.	.	.	32	15	7
10	Postages and Addressing	.	.	.	.	.	8	8	8
40	Reprints	.	.	.	.	.	30	13	6
	„ Other Expenditure—								
53	Library Books and Binding	.	.	.	.	.	3	17	9
33	Stationery, Printing, Postages and Sundry								
	Expenses	.	.	.	.	.	114	4	7
10	Repairs and Renewals of Furniture	.	.	.	.	.	5	8	3
9	„ Depreciation on Furniture	.	.	.	.	.			
	„ Balance of Income over Expenditure	.	.	.	.	.			

THE YEAR ENDED 31ST DECEMBER, 1946.

Gr.

1945.		INCOME.	£	s.	d.	£	s.	d.
£								
835	By Fellows Subscriptions		833	12	9			
417	„ Journal Subscriptions and Sales		391	0	5			
						1224	13	2
	„ Journal Advertisements, less discounts					28	12	3
	„ Income from Investments—							
	Gross amount		181	9	2			
50	Less : Income Tax deducted at source		37	16	6			
						143	12	8
2	„ Bank Interest					2	9	11
1	„ Other Receipts					7	1	
	„ Transfer from Superannuation Account					3	11	

£1399 19 0

„ Balance of Income over Expenditure b/d	20	18	2
„ Donations	9	10	0
„ Income Tax refunded three years to 5th April, 1945	117	10	4

£147 18 6

„ Balance b/d	147	18	6
„ Bad Debt reserve not required	252	3	9
„ Net excess of Debits over Credits	112	14	9

£512 17 0

Donations were reported from :—

Mrs. E. B. T. Ogilvy—

Microscope, by H. Powell, c. 1850 with accessories in Mahogany Case.

The Ray Society—

“The Trematoda of British Fishes,” by Ben Dawes, D.Sc., F.L.S.

Paper.—The following demonstration was given and discussed :—

Dr. C. R. Burch, F.R.S., demonstrated and described his reflecting Microscope.

He then called upon Mr. G. P. S. Occhialini, Dr. Robert Barer, B.Sc., and Mr. W. J. Bates to give further details of the work done in connection with this microscope.

The Lord Charnwood, F.R.M.S., also demonstrated his Dissecting Microscope which is being used in the Fenestration Operation for deafness.

Announcement.—The President made the following announcement :—

The Biological Section will meet on Wednesday, May 7th, 1947.

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, MAY 21ST, 1947, AT 5.30 P.M., DR. R. J. LUDFORD, PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed and signed by the President.

New Fellows.—The following Candidates were balloted for and duly elected Ordinary Fellows of the Society :—

T. Bennett.	London.
A. H. Edwards.	Chichester.
M. E. Folan.	Cork.
A. G. Sabin.	New Barnet.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner :—

A. Brecker.	London.
F. Galloway.	Leicester.
N. S. Macqueen.	Sevenoaks.
J. C. Meadley.	Manchester.
B. O. Payne.	Burnholme.
G. I. Wells.	Harrogate.
S. R. Gupta.	Allahabad.
R. W. Weeks.	London.
H. A. Burgess.	London.

Donations were reported from :—

Mr. D. J. Scourfield, *I.S.O.*, *F.L.S.*, *F.Z.S.*, *F.R.M.S.*—

4 Slides of *Daphnia Lumholzi* Sars.

Messrs. Chapman & Hall—

“The Intelligent Use of the Microscope,” by C. W. Olliver.

Papers.—There was a discussion on the work of the Cine-Micrographic Film Review Panel and a demonstration of the following films :—

“Movements of the Intestinal Villi.”

(Made by Professor Verzar of Basel.)

“Microscopical Observations of Living Tissues.”

(Made by R. H. Ebert, H. W. Florey, and A. G. Sanders.)

“The Microscope.”

(An instructional film on the use of the microscope with transmitted light.

Produced by the Realist Film Unit, directed by Alex Straffer and Denys Parsons.)

“Sperm Cell Development in the Grasshopper.”

(A German Phase-contrast Film.)

Dr. W. Loos demonstrated a phase-contrast microscope.

Announcement.—The President made the following announcements :—

The first Ordinary Meeting of the 1947–1948 Session will be held on Wednesday, October 15th, 1947, and the next Biological Meeting on Wednesday, November 5th, 1947.

The Proceedings then terminated.

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